Synthese und Struktur-Aktivitäts-Beziehungen von Flavonoiden

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Dipl.-Chem. Sophie PERRUCHON

aus Saint-Brieuc, Frankreich

Berichterstatter:	Prof. Dr. W.-D. Fessner
Mitberichterstatter:	Prof. Dr. H.-J. Lindner
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Sophie PERRUCHON

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M. H. BUCHHOLZ	Merck KGaA Darmstadt	Rapporteur
M. W.-D. FESSNER	Technische Universität Darmstadt	Directeur
M. C. MOINET	Université de Rennes 1	Directeur
M. W.PAULUS	Université de Rennes 1	Examinateur
M. H.-J. LINDNER	Technische Universität Darmstadt	Rapporteur

Chaque difficulté rencontrée doit être l´occasion d´un nouveau progrès.

"Every encountered difficulty should be the occasion of a new progress."

Baron Pierre de Coubertin
(1863-1937)

à Yves

17.07.1966-20.01.2004

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ABBREVATIONS

AA	antioxidant activity
ABTS	2,2′-azino-bis-(3-ethyl-benzothialin)-6-sulfonic acid
Ac	acetyl
AE	antiradical efficiency
AFO	Algar, Flynn and Oyamada reaction
AIBN	2,2′-azobisisobutyronitrile
Akt	protein kinase B
Ar	aryl
ATP	adenosine triphosphate
bp	boiling point
br	broad
cat	catalyst
c	concentration
cDNA	complementary deoxyribose nucleic acid
concd	concentrated
COSY	correlation spectroscopy
d	doublet
DMD	dimethyldioxirane
DMF	dimethylformamide
DMSO	dimethylsulfoxide
DNA	deoxyribose nucleic acid
DPPH	2,2-diphenyl-1-picrylhydrazyl hydrat
EC_{50}	efficient concentration at the steady state
equiv	equivalent
FAB-MS	fast atom bombardment mass spectrometry
h	hour(s)
HPLC	high performance liquid chromatography
Hz	Hertz
IC_{50}	inhibitory concentration at the steady state
IR	infrared
LCL	lower confident line
LDA	lithium diisopropylamide

LiHMDS	lithium bis-(trimethylsilyl)amide
m	multiplet
m-CPBA	*m*-chloroperoxybenzoic acid
min	minute(s)
MLR	multiple linear regression
mol	mole(s)
mp	melting point
mRNA	messenger ribose nucleic acid
NBS	*N*-bromosuccinimide
NCS	*N*-chlorosuccinimide
NMR	nuclear magnetic resonance
PCC	pyridinium chlorochromate
PCR	polymerase chain reaction
Ph	phenyl
PkB	protein kinase B
PTK	protein kinase
ppm	part per million
QSAR	quantitative structure activity relationship
RAS	Rapport Activité Structure
ROS	reactive oxygen species
rt	room temperature
s	singlet
SAR	structure activity relationship
t	triplet
TEAC	trolox equivalent antioxidant capacity
T_{EC50}	time at EC_{50}
Ti2	tyrosine kinase expressed in endothelial cells
THF	tetrahydrofuran
tlc	thin layer chromatography
TMS	tetramethylsilane
TTA	thallium (III) acetate
TTN	thallium (III) trinitrate
TTS	thallium (III) toluene-*p*-sulfonate
UCL	upper confident line

ABSTRACT

Flavonoids are natural polyphenolic compounds that are widely distributed in higher plants. Many of them possess biological activities (radical scavengers, anti-inflammatory...), which are potentially exploitable in the cosmetic field. Furthermore, flavonoids absorb in the UV range, which confers to them UV filter properties. In our laboratory, flavonoids and polyphenol derivatives were investigated to understand which part of the molecule is essential to provide the above mentioned activities.

Starting from a mono or polyhydroxylated aromatic compound, simple syntheses of known and new mono and polyphenol compounds have been developed to form a model compound library. By modifying the position of the hydroxyl groups around the benzene ring(s), it was possible to obtain molecules having interesting properties. Among them, the 7-hydroxy-4′-methoxyflavone absorbs not only in the UVB, but also in the UVA ranges with usefully high absorption coefficients.

Some of the synthesised molecules possess an ideal chemical structure for scavenging free radical. They have been shown to be more effective antioxidants *in vitro* than vitamins E on a molar basis. These results have been obtained by modifying either the substitution patterns around the core of the flavonoids or the oxidation level of the molecules (e.g. Bacalein). The biological effects of 7-*O*-glucosyl-4′-methoxyflavone were also tested using cDNA arrays containing a set of more than 400 genes related to skin functions. Selected compounds of the library were tested for their ability to inhibit protein-tyrosine kinases.

RESUME

Les Flavonoides sont des composés polyphénoliques largement distribués dans de nombreuses plantes. La plupart d'entre eux possèdent des activités biologiques (piégeur de radicaux, anti-inflammatoires …), très recherchées dans l'industrie cosmétique. En outre, les flavonoides absorbent dans la zone des ultraviolets, ce qui leur confère des propriétés de filtres UV. Dans notre laboratoire, les flavonoides et les dérivés polyphénoliques ont été étudiés pour comprendre quelle partie de la molécule est essentielle pour fournir les activités mentionnées ci-dessus.

Des synthèses simples à partir d'un cycle phényle mono- ou polyhydroxylé, ont permis de produire des composés mono- ou polyphénoliques, connus ou nouveaux, qui ont été rassemblés dans un modèle de bibliothèque. En modifiant la position des groupements hydroxyles autour du (des) cycle(s) benzénique(s), il a été possible d'obtenir des molécules très intéressantes. Parmi elles par exemple, le 7-hydroxy-4′-methoxyflavone absorbe dans la zone des UVB et des UVA avec un fort coefficient d'absorption.

Certaines des molécules synthétisées possèdent une structure chimique idéale en tant que piégeurs de radicaux libres et elles se sont révélées être des antioxydants plus efficace *in vitro* que la vitamine E, sur des bases molaires. Ces résultats ont été obtenus en jouant soit sur le motif des substitutions autour du corps des flavonoides, soit sur le degré d'oxydation des molécules (e.g. Bacalein). Les effets biologiques de la 7-*O*-glucosyl-4′-methoxyflavone ont également été testés utilisant des matrices d'ADN complémentaire, comprenant une série de plus de 400 gènes relatif aux fonctions de la peau. Des composés sélectionnés dans la bibliothèque ont été testés pour leur capacités d'inhiber les protéines tyrosines kinases.

ZUSAMMENFASSUNG

Flavonoide sind polyphenolische Verbindungen, die in zahlreichen Pflanzen weit verbreitet sind. Viele von ihnen besitzen biologische Eigenschaften (als Radikalfänger, Entzündungshemmer...), die möglicherweise auf kosmetischem Gebiet möglicherweise nutzbar sind. Flavonoide absorbieren beispielsweise im UV-Bereich, was ihnen gute UV-Filtereigenschaften verleiht. In unserem Labor wurden Flavonoide und polyphenolische Derivate untersucht um zu verstehen, welcher Teil des Moleküls für die obengenannten Eigenschaften verantwortlich ist.

Ausgehend von mono- oder polyhydroxylierten Aromaten wurden einfache Synthesen von bekannten und neuen, mono- und polyphenolischen Verbindungen entwickelt, die in einer Substanzbibliothek zusammengefasst wurden. Durch Veränderung der Hydroxylierungsposition an den Benzolringen, war es möglich Moleküle mit sehr interessanten Eigenschaften zu erhalten. Unter diesen absorbiert das 7-Hydroxy-4′-methoxyflavon sowohl im UVB-, als auch im UVA- Bereich mit sehr hohen Extinktionskoeffizienten.

Einige der synthetisierten Moleküle besitzen beste Voraussetzungen, um als Abfänger für freie Radikale zu wirken. Es könnte gezeigt werden, dass diese *in vitro* auf molarer Basis wirksamere Antioxidanzien sind als Vitamin E. Diese Ergebnisse wurden sowohl durch Variation des Substitutionsmusters um den Kern der Flavonoide als auch des Oxydationsgrads der Verbindungen erreicht (z.B. Bacalein).

Die biologische Wirkung von 7-*O*-Glucosyl-4′-methoxyflavon wurde daruberhinaus mit cDNA-Matrizen geprüft, welche eine Gruppe von mehr als 400 mit der Haut assoziierte Gene enthalten. Ausgesuchte Verbindungen der Musterbibliothek wurden außerdem darauf hin untersucht, inwieweit sie in der Lage sind Tyrosine-Kinasen zu hemmen.

INDEX

Introduction

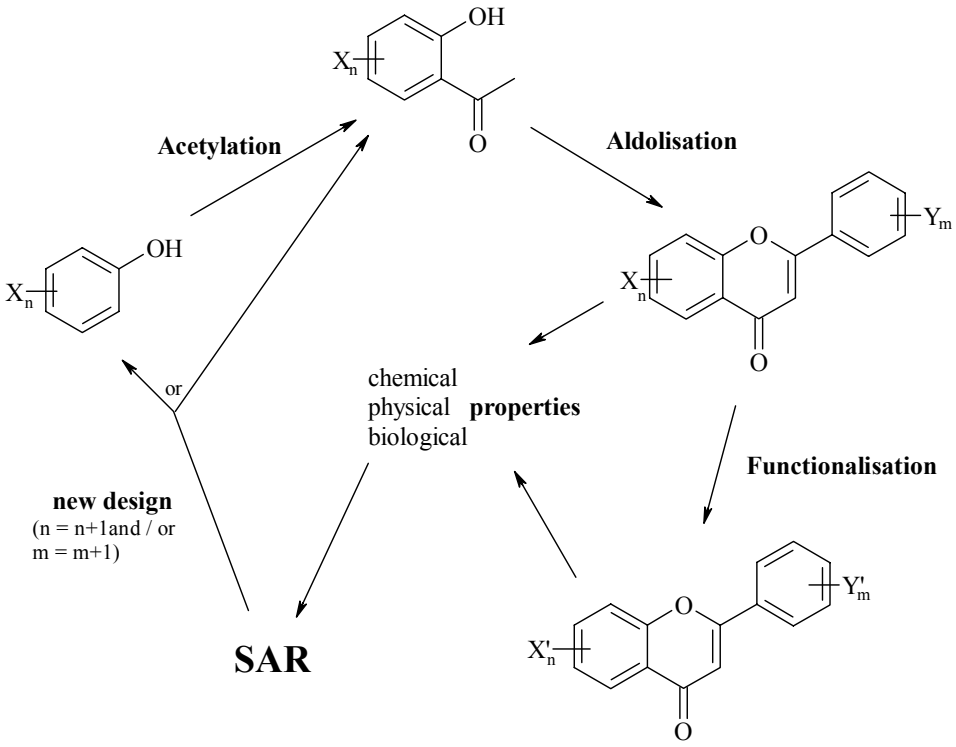
Flavonoids are natural products that are widely distributed in the plants kingdom. Their natural aspect and their various properties are very attractive for the pharmaceutical, cosmetic and nutrition fields of application. The isolation of bioflavonoids is carried out *via* an extraction process, but usually the plants contain a variety of derivatives in low concentration. Thus, large amounts of dried raw material and laborious chromatography purification schemes are needed to isolate quantities of individual compounds.

The synthesis of bioflavonoids represents a challenge for chemists, in terms of multi-step synthesis and regioselective modifications of polyphenols. Even if many syntheses are available to build the flavonoid aglycones[*], those methods are often limited by the substitution patterns of the precursors.

The first goal of this work was to develop new routes to known and new flavonoids (focused on flavones), using a minimum of steps and inexpensive starting materials. The second goal was to functionalise the synthetic flavonoids *via* chemical transformations to study the variation of the physical, chemical and biological properties in a systematic Structure-Activity Relationship (SAR; Scheme 1).

Among all the available methods, chalcones and diketones were frequently registered as intermediates of flavonoid syntheses, which show that the aldolisation is the key step in flavonoid synthesis. Thus, an investigation of aldolisations with a minimum of protection requirement should be carried out in order to obtain polyhydroxylated flavonoids, taking in account the competition of reactivity of the hydroxyl groups.

[*] Flavonoid free from any sugar moiety



$X = (OH)_{n=0,1,2,3}, (OCH_2O)_{n=0,1}$; $Y = (OCH_3)_{m=0,1,2,3}, (OCH_2O)_{m=0,1}, (Cl)_{m=0,1}, (NO_2)_{m=0,1}$
$X' = OH$, O-Alkyl, O-glucosyl $_{n=0,1,2,3}$; $Y' = OH, NH_2$ $_{m=0,1,2,3}$

Scheme 1

The results of the SAR study (UV absorption, antioxidant activity, protein tyrosine kinase inhibitor ability) should provide enough information to design and synthesise new acetophenones or phenols in order to obtain the corresponding attractive flavonoids. Ideally, the synthesis strategy should be applicable to other, structurally related flavonoids with only minor adjustments, for individual substitution patterns.

PART I

Chapter 1: General Informations

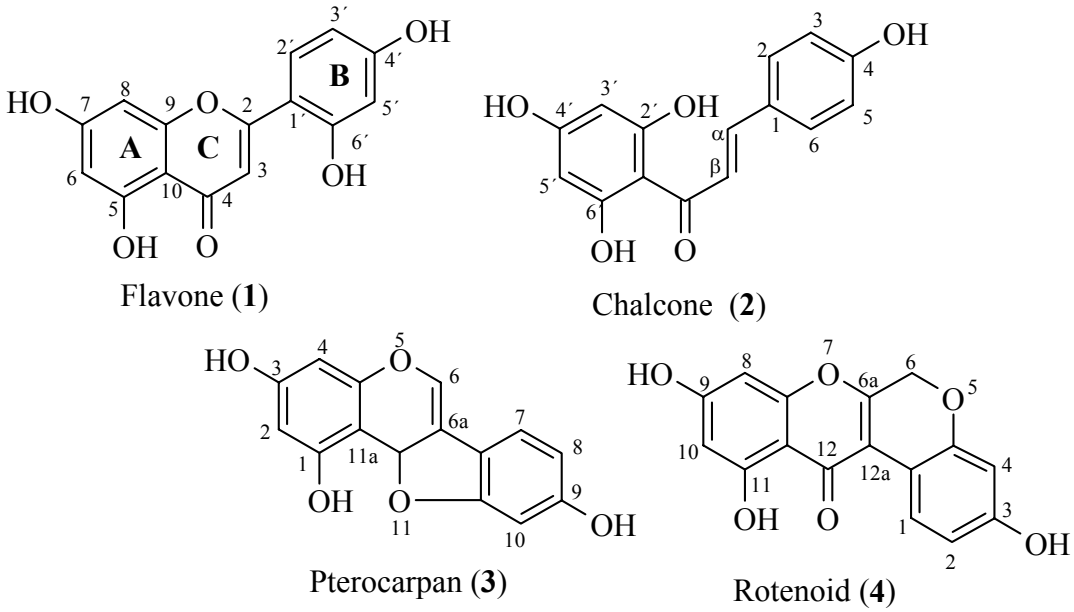
1. General introduction to the flavonoids

1.1. Definition

The Flavonoids are a very large and important group of polyphenolic natural products, which are united by their derivation from the fused aromatic heterocyclic ring system, (2-phenylbenzopyranone) commonly named flavone (**1**). It occurs naturally as farina on *Primula* plants. Some flavonoids are intensely coloured, e.g. the anthocyanins providing a wide range of red to blue colours in flowers, fruits and leaves. Others, like the flavones, are essentially colourless and yet they provide the whiteness of white flowers and act as co-pigments to the widespread anthocyanins.

1.2. Structures

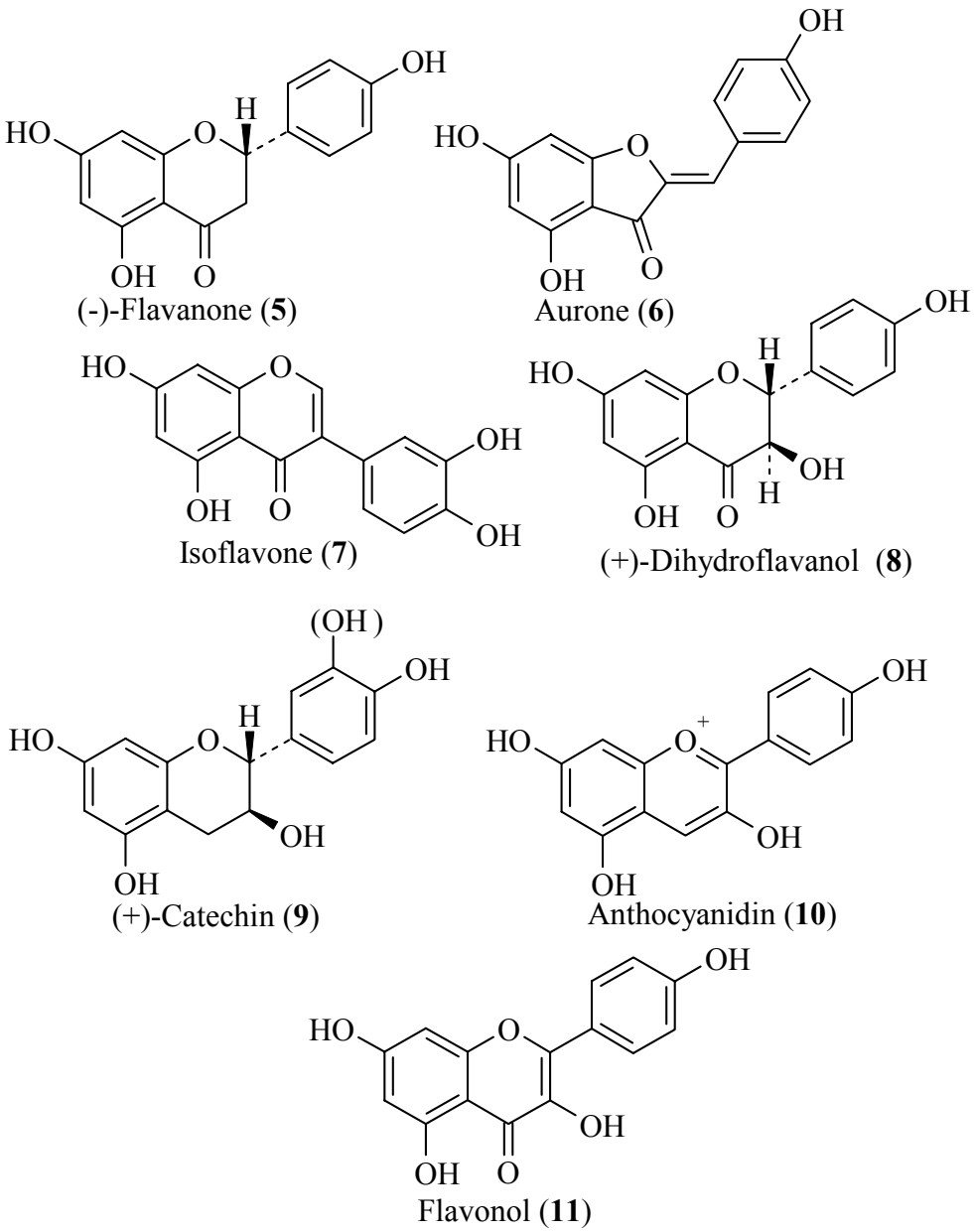
In plants, flavonoid aglycones (i.e. flavonoids without attached sugars) occur in a variety of structural forms. All contain fifteen carbon atoms in their basic nucleus: two six membered-rings linked with a three-carbon unit which may or may not be a part of a third ring. For convenience the rings are labelled A, B, and C. The individual carbon atoms are based on a numbering system (Scheme 2), which uses ordinary numerals for the A- and



Scheme 2: Numbering system of flavonoids

C-rings and “primed” numerals for the B-ring. Primed modified numbering system is not used for the chalcones (**2**), and the isoflavones derivatives: the pterocarpans (**3**) and the rotenoids (**4**).

The different ways to close this ring associated with the different oxidation degrees of ring A provide the various classes of flavonoids (Scheme 3): flavones (**1**), pterocarpans (**3**), rotenoids (**4**), flavanones (**5**), aurones (**6**), isoflavones (**7**), flavanols (**8**), catechins (**9**), anthocyanins (**10**) and flavonols (**11**).



Scheme 3: Examples of different varieties of flavonoids

Flavonoids may also be classified according to molecular size. While the majority is monomeric, a significant number of dimeric, trimeric, tetrameric, and polymeric structures have been described. Most biflavonoids (or biflavonyls) are based on carbon-carbon linking of two similar flavone units, but mixed dimers are also known (e.g. flavonylflavanones). The highest molecular weight flavonoids are the oligomeric and polymeric proanthocyanidins, derived synthetically from flavan-3-ols. They consist of 4 to 8 linked flavan units, either as straight chains or with side branching through other links.

Most flavonoids occur naturally associated with sugars in conjugated form and within any one class may be characterized as monoglycosidic, diglycosidic, etc. Glycosidic complexity is considerable and monosaccharides associated with flavonoids include glucose, galactose, arabinose, rhamnose, xylose, apiose, allose, mannose, galacturonic acid, and glucuronic acid. Mono-, di- and tri-saccharides may be linked through a single phenolic hydroxyl or may be variously linked to two or more phenolic groups. Acylated *O*-glycosides have aromatic (e.g. *p*-coumaric) or aliphatic (e.g. malonic) acids linked, usually through the 6-hydroxyl, to the glucose moiety. A special group of mainly flavone-based *C*-glucosides occurs in plants and those may additionally be present as *O*-glycosides as well. Sulfated conjugates are common in the flavone and the flavonol series, where sulfation may be on a phenolic hydroxyl and/or on an aliphatic hydroxyl of a glycosidic moiety.

1.3. Nomenclature

In general, there are two parallel systems of flavonoid nomenclature, one based on trivial names, such as flavan and chalcone, as the parent structure and the other based on systematic chemical name, such as 3,4-Dihydro-2-phenyl-2*H*-1-benzopyran (IUPAC nomenclature) for flavans and 2-phenyl-4-oxo-*4H*-1-benzopyran for flavone. The latter becomes cumbersome, and is easy to get wrong in cases of polysubstitution that is why it is rarely used. There are also two systems of ordering the substituents around the flavan nucleus; one in which the A-

and C-ring substituents precede B-ring substituents (e.g. 3,5,7,3′,4′-pentahydroxyflavone, quercetin) and one in which the substituents are ordered numerically (e.g. 3,3′,4′,5,7-pentahydroxyflavone). There are additionally two conventions for drawing formulae, with the heterocyclic oxygen at the top or at the bottom.

Trivial names are employed extensively in the flavonoid literature, so that they are used widely here as well. Some names indicate the class of compound. For example the ending "inidin" denotes an anthocyanidin (e.g. pelargonidin), while the ending "etin" denotes a flavonol (e.g. quercetin). Certain glycosides of quercetin have related names: quercitrin, the 3-rhamnoside; isoquercitrin, the 3-glucoside; quercimeritrin, the 7-glucoside. However, there is little consistency in the naming of flavonoids and many names are derived from the generic or specific name of the plant source (e.g. tricin form *Triticum*, hypolaetin from *Hypolaena*, etc.).

1.4. Distribution in the nature

Flavonoids in general are universally distributed in higher plants (Picture 1-3). They also occur in many lower plant groups, notably in mosses and liverworts. They have even been detected rarely in fungi, but there are no records so far from the bacteria. Due to the interest resulting particularly from the conspicuous vivid and beautiful colours these pigments impart

Picture 1
Antirrhinum spp (Scrophu-lariaceae) contains acacetin

Picture 2
Pterocarpus marsupium (Heartwood) contains apigenin

Picture 3
Pimelea decora (Thyme-laeaceae) contains 6-hydro xy-4′-methoxyflavone

to various parts of plants, flavonoids have been extracted from leaves[1,2] roots,[3] wood, bark,[4] pollen, nectar, flowers,[5] berries,[6] fruit skin or peel,[7] and seeds[8,9]. They have been detected during the fermentation of tea,[10,11] and the manufacture of cocoa[12].

1.5. Properties

Besides their contribution to plant colour, flavonoids have a variety of other roles in the growth and development of plants. There is evidence, for example, that leaf flavonoids, deposited either in the epidermal cells or in the waxy upper leaf surface, provide protection[13] from the potential damage of UV-B radiation. In addition, it has been discovered that the flavones of legumes roots have an essential signalling role in the process of infection by *Rhizobium* bacteria and hence in the establishment of nitrogen fixation in these plants. Physiological experiments in Petunia flowers indicate that the flavonol glycosides secreted in plant pollen play a part in the reception of the pollen on the plant stigma and are required for successful reproduction.

Flavonoids exhibit a wide rang of biological properties, including antibacterial,[14,15] insecticidal[16] and estrogenic,[17,18] activities. For example, certain flavanones and isoflavanones are formed *de novo* as antifungal barriers in the plant leaves, in response to microbial infection; other plant flavonoids provide constitutive antifungal resistance. There is much evidence that flavonoids are involved in plant-animal interactions and that they can have a role in the plant's defensive protection mechanisms. The flavolans or condensed tannins, in particular, have the ability to bind with protein and, when present in the plant in high concentration, as oak leaves, defend the tissue from animal feeding.

Flavonoids occur widely in the fruits and vegetables that make up the human diet and it has been estimated that at least one gram of flavonoid is taken in daily. A lot of clinical studies and medications based on flavonoids are beneficial to human health. Indeed there is some evidence of antimutagenic,[19,20] antioxidant,[21,22] and radical-scavenging properties.

Flavonoids are also major components of many plant drugs (e.g. ginkgo leaves) and it is possible that they contribute to the curative properties. Many flavonoids are pharmacologically active, and some have useful anti-inflammatory[23,24] antiviral,[25,26] antineoplastic*,[27] antithrombotic†,[28,29] vasodilatory,[30,31] or hepatoprotective properties.

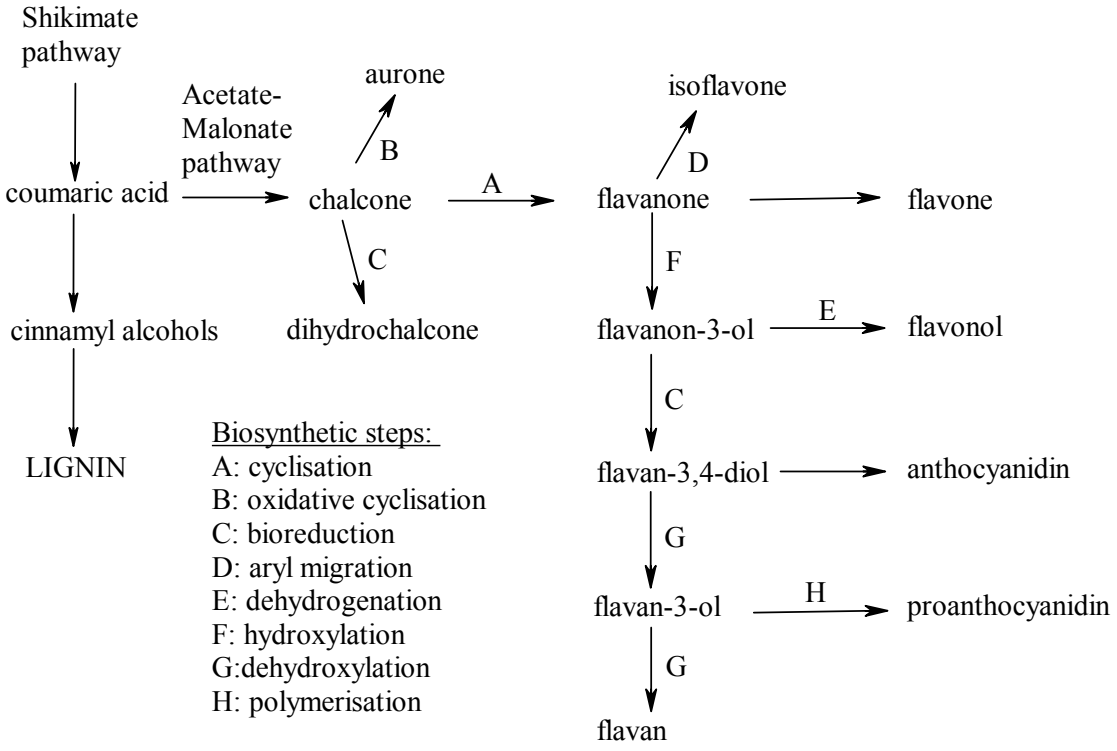
2. Syntheses

2.1. The biosynthetic pathway

A common biosynthetic pathway (Scheme 4) relates to all the flavonoid variants that incorporate precursors from both the "Shikimate" and "Acetate Malonate" pathways,[32,33] the first flavonoid initially formed in the biosynthesis is the chalcone[34] and all other forms are derived from this variety of routes. Further modification of the flavonoid may occur at various stages resulting in: additional (or reduced) hydroxylation; methylation of hydroxyl groups or of the flavonoid nucleus; isoprenylation of hydroxyl groups or of the flavonoid nucleus; Methylenation of *ortho*-dihydroxyl groups; dimerisation (to produce biflavonoids); bisulfate formation; and most importantly, glycosylation of hydroxyl groups (to produce flavonoid *O*-glycosides) or of flavonoid nucleus (to produce flavonoid *C*-glycosides); the range of known flavonoids is thus vast and lists of variants have been published[35] and recently updated[36].

A wide range of enzymes catalysing steps of flavonoid biosynthesis have so far been identified, from the pathway to precursors (Acetyl-CoA Carboxylase (ACC),[37] Shikimatearogenate pathway,[38,39,40] Phenylalanine ammonia-lyase,[44] Cinnamate 4-hydrolase)[41] to the major steps of the flavonoid pathway (Chalcone synthase,[44,42] formation of the 6′-deoxychalcone,[43,44,45] flavone Synthases (FNS I and II),[44,46,47] flavanone 4-reductase (FNR),[48,49] flavanone 3-hydrolase (FHT),[44,50,51] flavonol synthase (FLS),[44,52,53] dihydroflavonol 4-reductase (DFR),[44,54] Catechin and proanthocyanidin formation,[55,56,57,58] and the conversion of flavan-3,4-diol into anthocyanidin 3-*O*-glucoside).[44,59]

* antineoplastic: said of a drug intended to inhibit or prevent the maturation and proliferation of uncontrolled cell growth that may become malignant by targeting DNA
† antithrombotic: anticoagulant



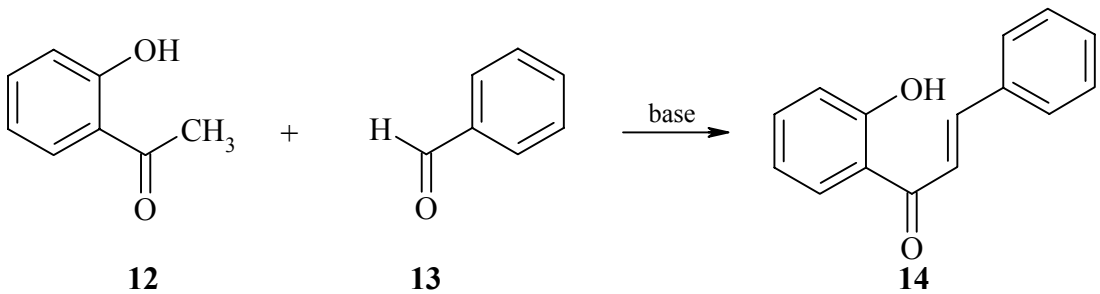
Scheme 4: Currently proposed interrelationships between flavonoids (supported by varying levels of experimental evidence) [40] for the Biosynthetic pathways

2.2. Chemical syntheses

2.2.1. The most important intermediates to obtain

2.2.1.1. Chalcones

2′-Hydroxychalcones (**14**) are the most important intermediates for the synthesis of flavonoids[60] like flavones, flavonols, 3-hydroxyflavanones and aurones. The formation of chalcone involves the Claisen-Schmidt-Condensation of an aromatic aldehyde (**13**) with an acetophenone (**12**) in the presence of alkali as catalyst (Scheme 5).

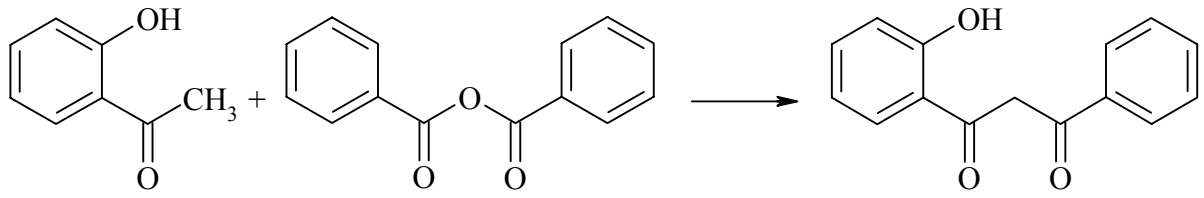


12 **13** **14**

Scheme 5: Condensation into a chalcone (**14**)

2.2.1.2. β-Diketones

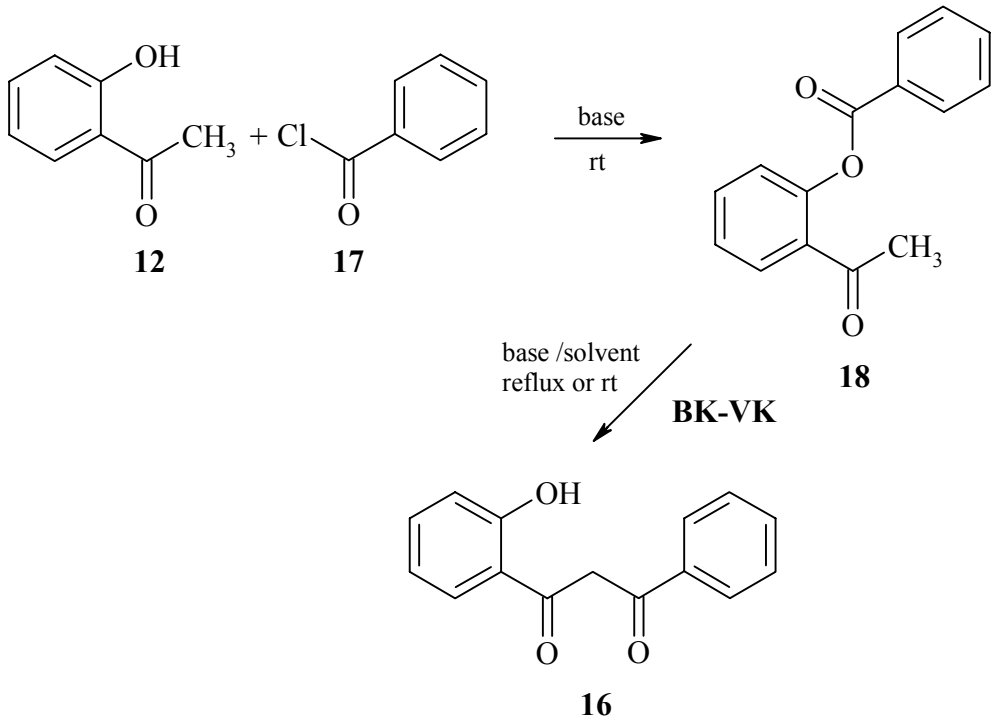
β-Diketone or 1-(2-phenyl)-3-phenylpropane-1,3-dione (**16**) is considered as a critical intermediate for the synthesis of flavones (Scheme 6). β-Diketones are obtained via the Allan-Robinson[61] reaction which involves the condensation of an acetophenone (**12**) with a benzoic acid anhydride (**15**).



12 **15** **16**

Scheme 6: Condensation into a β-diketone (**16**)

Alternatively, β-diketone (**16**) can be prepared in two synthetic steps using the Baker-Venkataraman reaction (BK-VK)[62] shown in Scheme 7. It is the conversion of a 2-hydroxyacetophenone (**12**) to a benzoylester (**18**), which is treated with a base to induce an intramolecular Claisen condensation forming a 1,3-diketone (**16**).



12 **17** **18** **16**

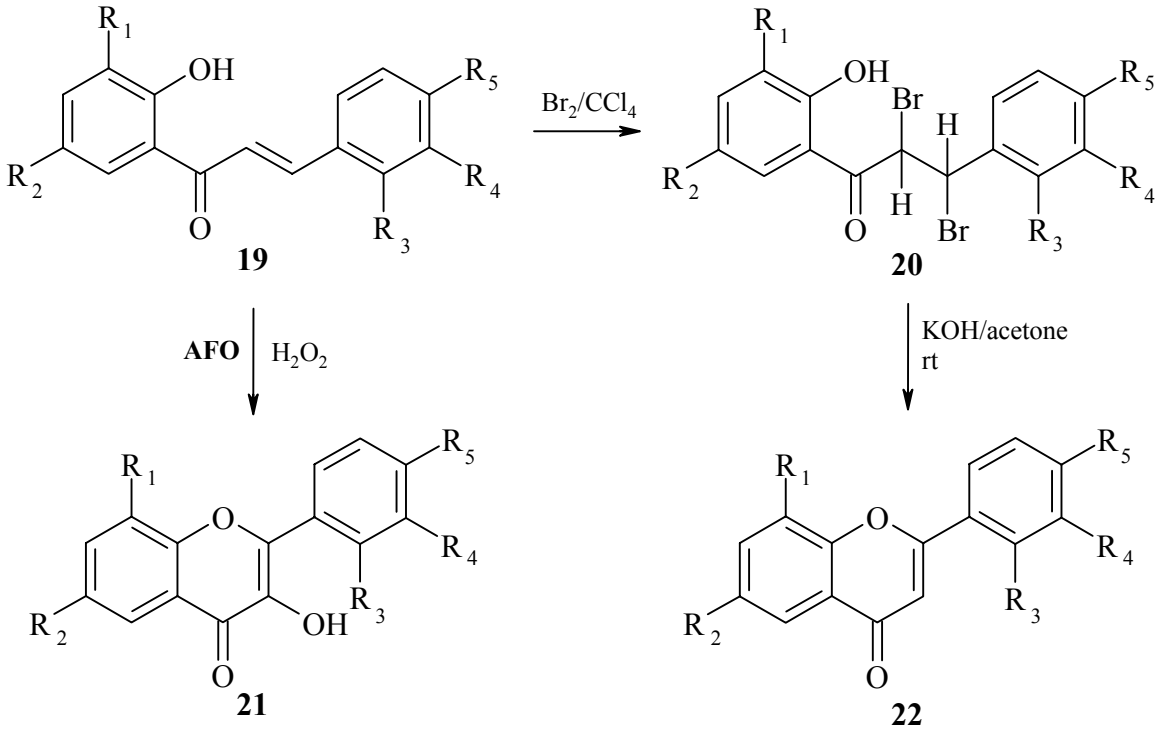
Scheme 7: Conventional Baker-Venkataraman rearrangement

The Baker-Venkataraman reaction was studied mainly with an ester under a great variety of experimental conditions using different bases such as KOH[63], K_2CO_3[64], NaOH[65], KO^{t}Bu, NaH[66], LDA[67], LiH[67], LiHMDS[67] (and mixtures thereof) in different amounts and solvents (DMF, THF, acetone) at different temperatures and reaction times.

2.2.2. Cyclisation of the intermediates

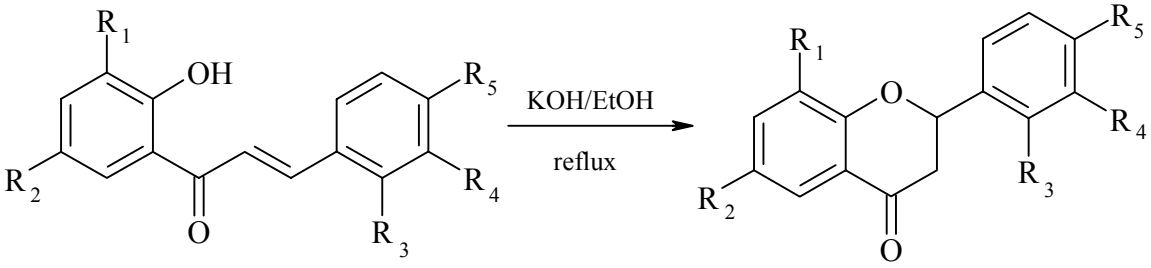
2.2.2.1. Chalcones

Starting from a chalcone (**19**), many different methods have been used to cyclise it and thus access to various types of flavonoids. These methods imply the use of acids,[68] bases,[69] silica,[70] light,[71] Co (II) Schifft base complexes,[72] palladium[73] or platinum[74] salt, heat,[75] electrolysis,[76] and nickel chloride/zinc/potassium iodide reagents system.[77] One of the first methods, the bromination of chalcone (**19**) followed by the treatment of the resulting dibromides (**20**) with potassium hydroxide[78] gave the flavone (**22**), when the chalcone (**19**) was subject to the Algar Flynn Oyamada (AFO) reaction,[79,80] an alkaline hydrogen peroxide oxidation, the corresponding flavonol (**21**) was obtained (Scheme 8).



Scheme 8: Cyclisation of chalcone (**19**)

The cyclisation of the 3′-phenylchalcone (**19**) in alcoholic medium and base also leads to the corresponding 6- and 8-substituted flavanones (**23**) (Scheme 9). Furlong and Nudelman[81]



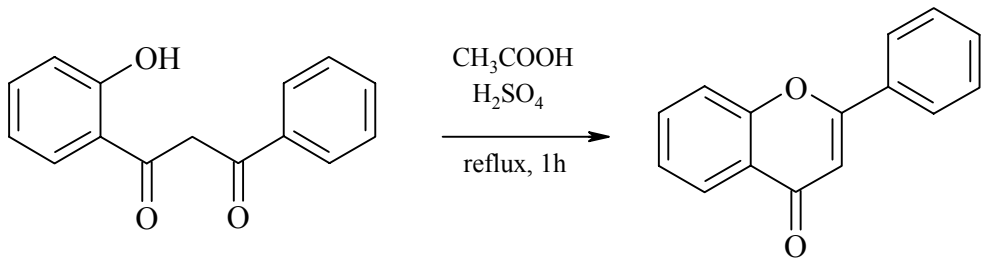
19 **23**

Scheme 9: Cyclisation of chalcone (**19**) into flavanone (**23**)

confirmed a mechanism for this step, which involves general acid attack of the ionised form of the 2′-hydroxychalcones, concerted rotation through the CO-C_α bond, and annellation to the flavanone.

2.2.2.2. *β*-Diketones

The Cyclisation of the *β*-Diketone (**16**) can be carried out in acetic acid with a catalytic presence of sulphuric acid (0.5%v) by heating between 30 minutes to one hour (Scheme 10). After evaporation of 75% of the solvent, the work up in water usually affords a precipitated flavone (**24**).



16 **24**

Scheme 10: Cyclisation of the *β*-diketone (**16**)

The use of ethanol instead of acetic acid showed no significant changes or improvements.

2.3. Other syntheses

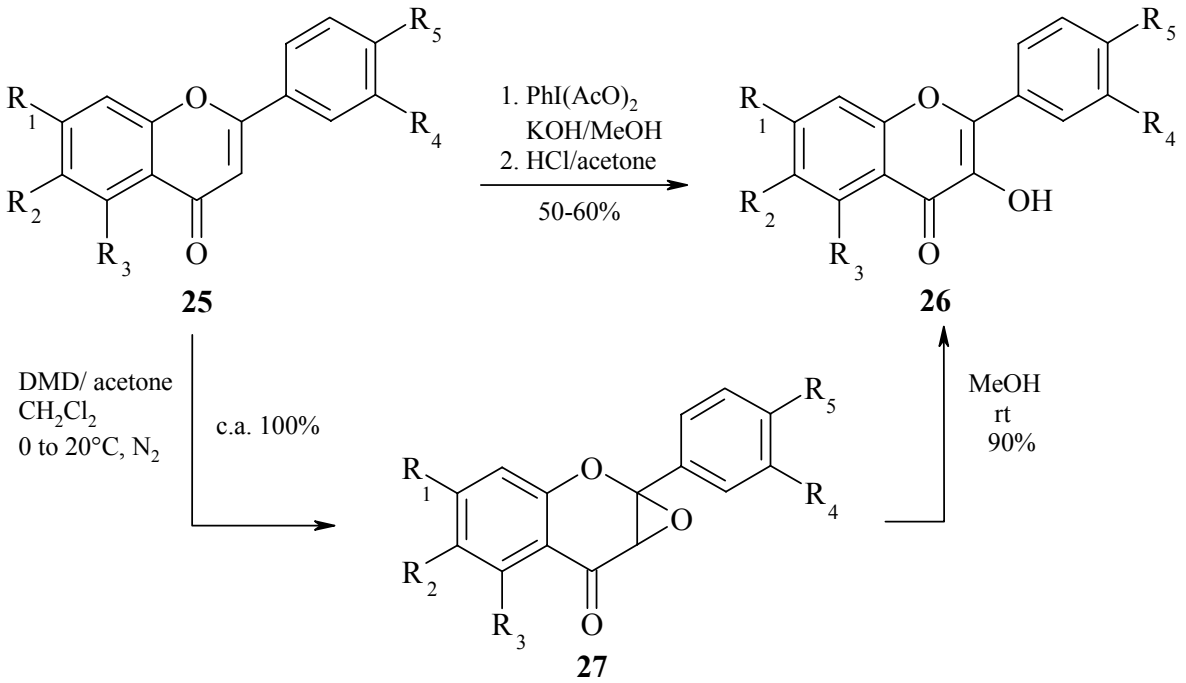
2.3.1. On flavonoids

Many authors have been worked on extracted or available flavonoids to transform them

into other classes of flavonoids.

2.3.1.1. Epoxidation of flavones

The conversion of flavones (**25**) to their epoxides (**27**), potentially useful intermediates for synthetic purposes, by classical oxidants H_2O_2,[82] m-CPBA,[76] SeO_2,[83] $KMnO_4$[84] or NiO_2[85] proved to be difficult. PhI(OH)(OTs)[86] oxidized a flavone (**25**) to a flavonol (**26**). The most convenient and efficient oxidant to carry out the epoxidation of flavone was the dimethyldioxirane (DMD) (Scheme 11).[87] In the synthesis of flavonols, epoxides (**27**) can easily be converted into the corresponding flavonols (**26**).

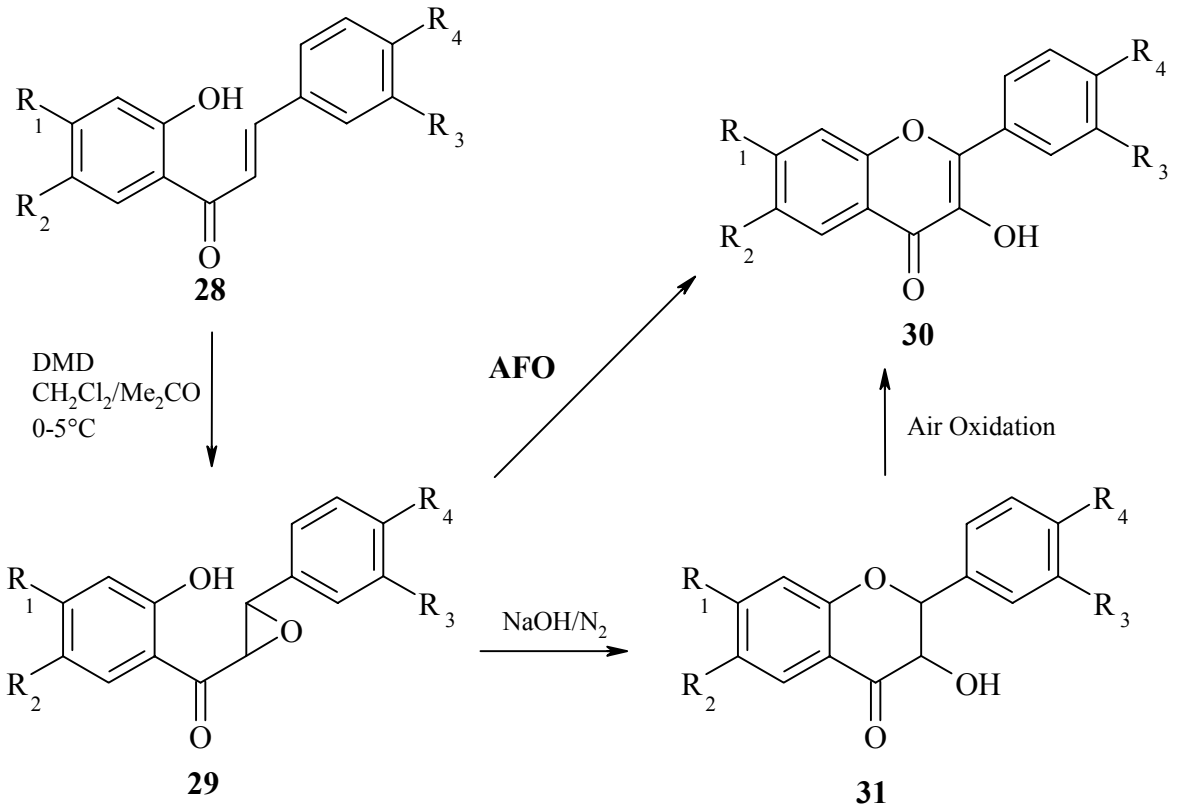


Scheme 11: Epoxidation of flavones

2.3.1.2. Epoxidation of Chalcones

The applications of DMD as the reagent of choice entail the epoxidation of electron-rich alkenes such as chalcones (**28**) has been investigated to afford another family of flavonoids: the flavanonols (**31**) (Scheme 12). Unlike the flavone epoxides, 2′-hydroxychalcone epoxides (**29**) are likely to be unstable near and above the neutrality. As a consequence the isolation of these intermediates proved quite difficult because of the facile cyclisation to flavanonol (**31**). An alkali treatment of the 2′-hydroxychalcone epoxides (**29**) converted them first into **31** and

then into **30**, as reported in the AFO reaction.[73,74] This undesired reaction can be minimized upon treatment with alkali in deoxygenated solution, which leads to the instantaneously isomerisation.[88]



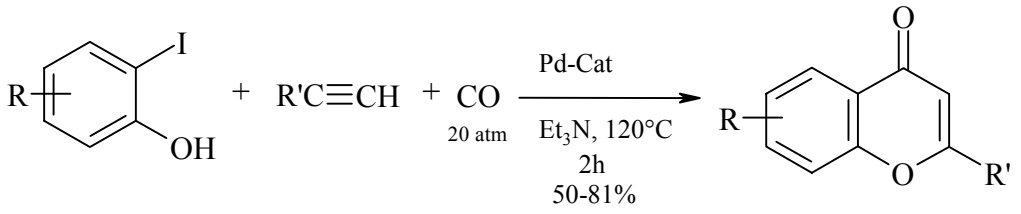
Scheme 12: Epoxidation of chalcone (**28**)

The reduction[89] of flavanones performed with sodium borhydride gave the corresponding flavan-4-ols, while the oxidation of flavanones or chalcones by DMSO-I_2[90] in catalytic amount gave the corresponding flavones in more than 80% in a convenient short time. Other authors used thallium salts (Thallium (III) acetate (TTA), Thallium (III) trinitrate (TTN) and Thallium (III) toluene-*p*-sulfonate (TTS)), versatile reagents in organic synthesis, that oxidize flavanones[91] or flavyliums salts[92] to flavones.

2.3.2. From other precursors

Palladium catalysed synthesis of flavones (**34**) has been reported[93] as a reaction of *o*-iodophenols (**32**) with terminal acetylenes (**33**) under carbon monoxide in the presence of secondary amine and catalytic amount of palladium complex, to be a convenient preparative

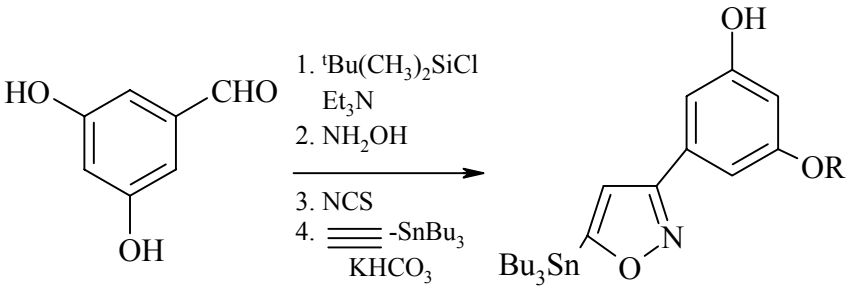
method (Scheme 13).



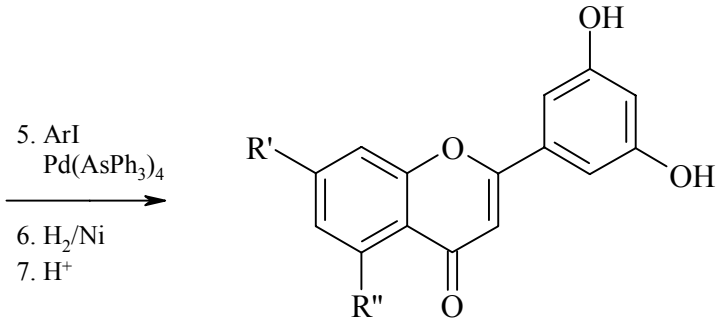
32 **33** **34**

Scheme 13: Palladium catalysed synthesis of flavones

Ellemose[94] and co-workers used isoxazole (**36**) protocol and Heck-Stille coupling to investigate the behaviour of *O*-silylated phenolics as reaction partners in these reactions and devised routes to polyhydroxylated flavones (**37**) in 11-84% (Scheme 14).



35 **36** R = $^{t}Bu(CH_3)_2Si$

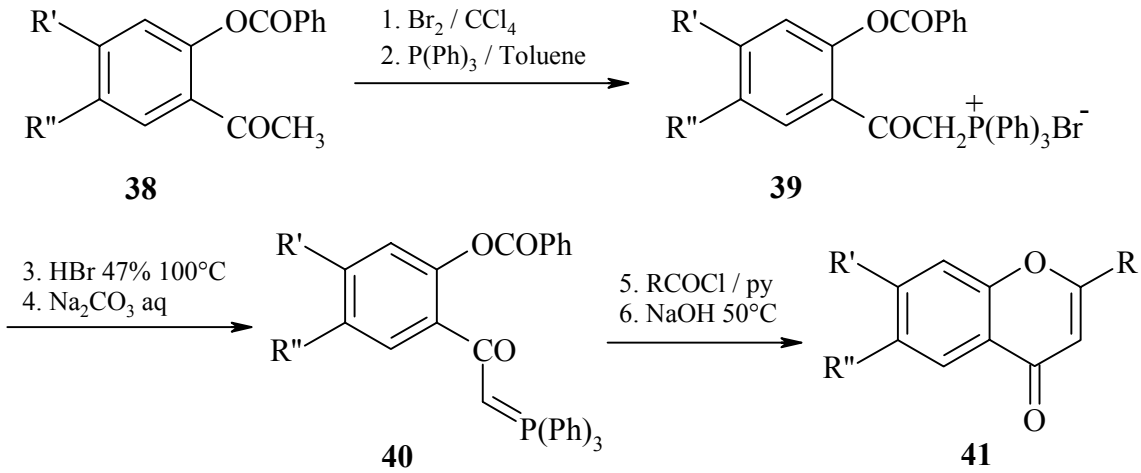


37

R', R" = H or OH

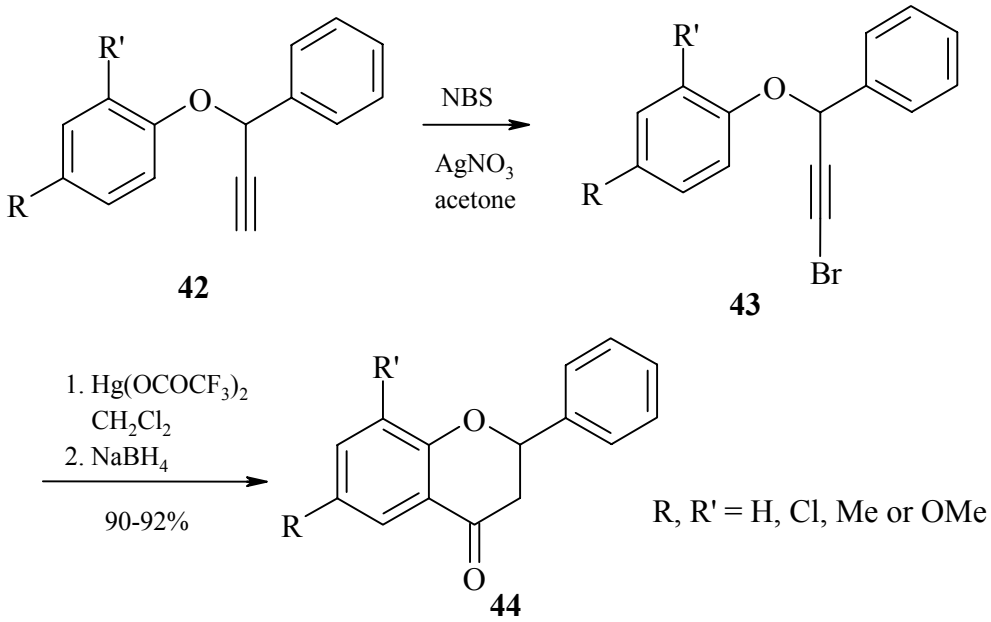
Scheme 14: Isoxazole methodology and Heck-Stille coupling

The synthesis of dihydroxyphenacylidene triphenylphosparanes from 2,4- or 2,5-dibenzoyloxyacetophenone (**38**) is described by Le Floc'H and Lefeuvre[95]. Acylation of the hydroxy groups of these ylids, followed by intramolecular olefination of the ester carbonyl groups and hydrolysis of the second ester group, affords some 6- or 7-hydroxychromones (**41**) (Scheme 15).



Scheme 15: Synthesis of ylids to chromones

Subramanian and Balasubramanian devised[96] the trifluoroacetate-mediated transformation to afford flavanones. The *γ*-bromo compounds (**43**) were prepared by the reaction of **41** with NBS in the presence of silver nitrate. The ethers underwent a facile transformation to

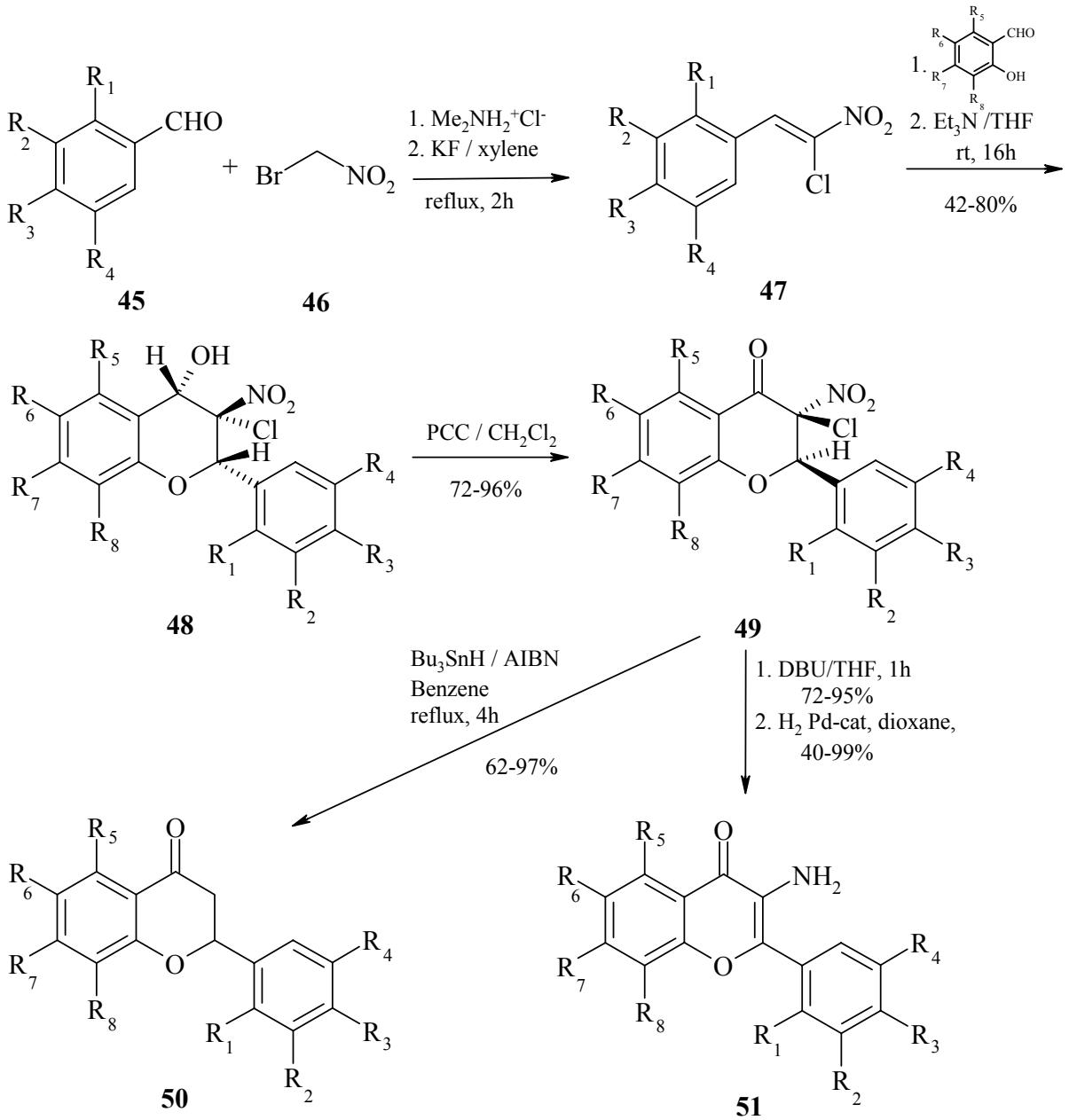


Scheme 16: Mercury (II) trifluroracetate-mediated transformation

flavanones (**44**) in the presence of mercury (II) trifluoroacetate after reductive work-up (Scheme 16).

An alternative synthesis of polyhydroxyflavanones can be carried out from the di- / trihydroxyacetophenone and mono- / dihydroxybenzaldehyde in the presence of boric acid[97] in a mixed solvent system.

The condensation of bromonitromethane (**46**) with benzaldehydes (**45**) led[98] to (2-chloro-2-nitroethenyl)-benzenes that further reacted with salicyl aldehydes to provide the substituted

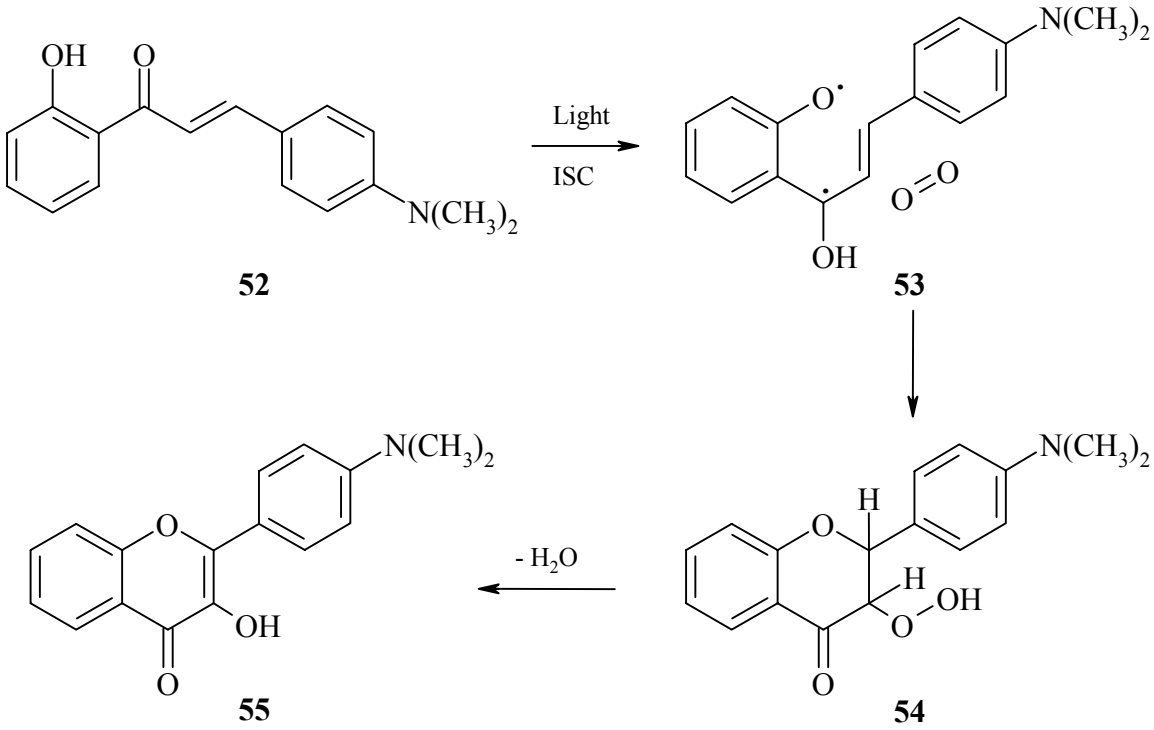


Scheme 17: Routes to flavavones (**50**) and 3′-aminoflavones (**51**)

dihydroxybenzopyrans (**48**). Subsequent oxidation of these compounds by pyridinium chlorochromate (PCC), gave[99] the oxoderivates (**49**) which were converted into desired either 3-nitroflavones upon basic treatment, and that could be reduced to 3-aminoflavones (**51**) in the presence of catalytic amount of palladium on activated carbon (10%), or flavanones (**50**) in the presence of 2,2′-azobisisobutyronitrile (AIBN) (Scheme 17).[100]

2.3.3. Photochemical synthesis

Brack and co-workers[101] studied the photochemical reaction on 4′-*N,N*-dimethylamino-2′-hydroxychalcone (**52**) in hydrocarbon solutions that leads to the formation of 4′-*N,N*-dimethylamino-3-hydroxyflavone (**55**) (Scheme 18).



Scheme 18: Photochemical reaction of the 4′-*N,N*-dimethylamino-2′-hydroxychalcone (**52**)

3. Quantitative Structure Activity Relationship (QSAR)

3.1. Definition

Ever since scientists began to measure, or quantify, the physical and biological properties of the natural world, they also sought patterns or relationships between the measurements they made. However, it was not until the 1930′s that knowledge of the extent and the rates of chemical processes, together with the properties of the reacting molecules (shape, size and electronic properties) allowed correlations to be made between the nature of molecules and their tendency to react.

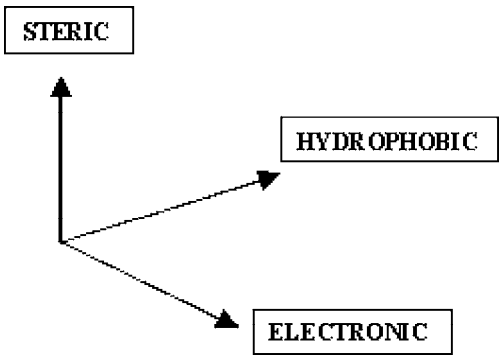
Surprisingly, even though similar types of measurements were possible in the biological world, particularly related to drug potency and toxicity, very few attempts were made to

connect biological activity and physical properties. Then, in the 1950′s, Hansch[102,103] developed a hydrophobic parameter and used regression analysis to correlate biological activity with molecular properties. Since then, scientists have used more sophisticated statistical methods and developed the use of other forms of pattern recognition, such as cluster analysis, factor analysis and principal components analysis, in the search for patterns between biological and physical data.

The aim of Quantitative Structure-Activity Relationship (QSAR) techniques[104,105] is to develop correlations between any property or form of activity, frequently biological activity, and the properties and usually physicochemical properties, of a set of molecules, in particular substituents properties. However, in its most general form, QSAR has been adapted to cover correlations independent of actual physicochemical properties. QSAR started with similar correlations between chemical reactivity and structure. Ideally, some known mathematical function, F, connects the activities and properties:

Biological activity = F (Physicochemical Properties)

Biological activity can be any measure such as C, IC_{50}, and EC_{50}.



Scheme 19: Orthogonality of the physicochemical types

Physicochemical properties can be broadly classified into three general types:

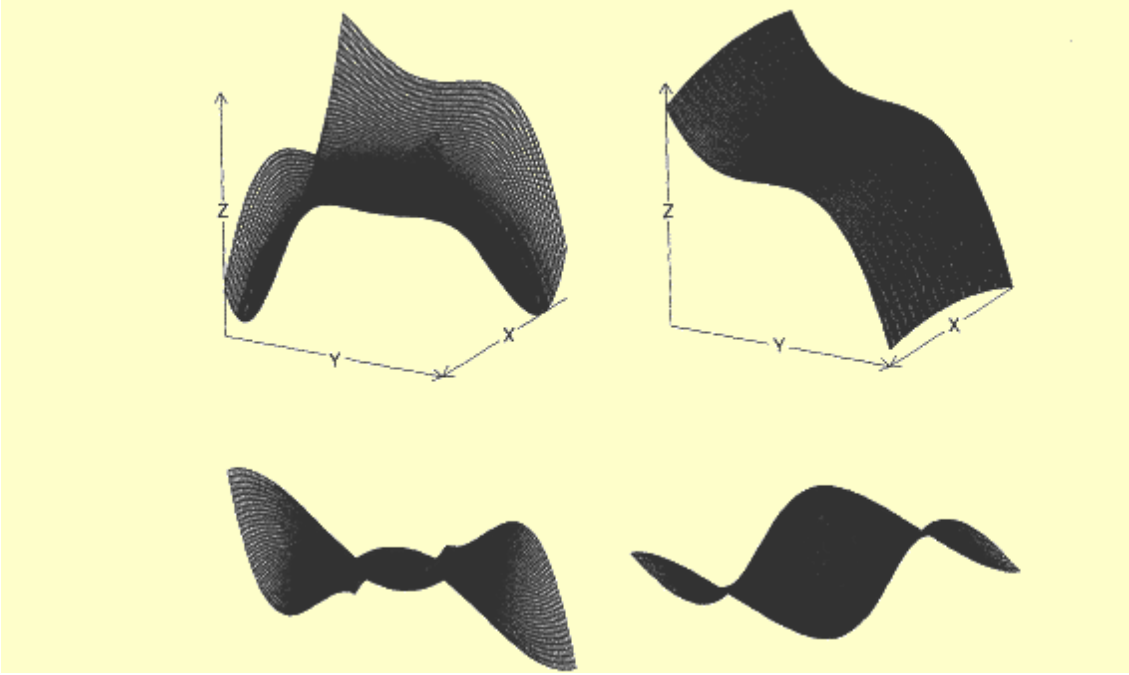
- Electronic

- Steric
- Hydrophobic

for which an enormous range of properties and physicochemical parameters have been defined. Ideally the parameters selected should be orthogonal, that is, have minimal covariance (Scheme 19).

The relationship, or function, is usually (but not always) a mathematical expression derived by statistical and related techniques, for example, multiple linear regression (MLR). The parameters describing physicochemical properties are used as independent variables and the biological activities are dependent variables. In some cases a function cannot be found, and this reflects the multi-variant, non-linear nature of biological and physical properties. Use of such data may be possible using neural networks to deduce essential data for activity and then using this for prediction.

Complex polynomial surfaces[106] that are capable of being derived using artificial neural networks (Scheme 20).



Scheme 20: Examples of complex polynomial surfaces.

Methods are also divided into unsupervised and supervised learning techniques. Unsupervised learning (e.g. cluster analysis, factor analysis) does not make use of a dependent variable, as is the case in supervised learning (e.g. regression analysis) in which a rule is deduced usually by using an algorithm that correlates independent and dependent variables.

Usually some data are used to generate a relationship (the training set) while a set of data is reserved as a test set on which predictions using the rule are made. In this way a model can be tested for validity. The complete range of techniques used to derive functional relationships between the data is collectively known as chemometrics.

3.2. The advantages and disadvantages of QSAR

3.2.1. Advantages of QSAR

- Quantifying the relationship between structure and activity provides an understanding of the effect of structure on activity, which may not be straightforward when large amounts of data are generated.
- There is also the potential to make predictions leading to the synthesis of novel analogues. Interpolation is readily justified, but great care must be taken not to use extrapolation outside the range of the data set.
- The results can be used to help understand interactions between functional groups in the molecules of greatest activity, with those of their target. To do this it is important to interpret any derived QSAR in terms of the fundamental chemistry of the set of analogues, including any outliers.

3.2.2. Disadvantages of QSAR

- False correlations may arise through too heavy a reliance being placed on biological data, which, by its nature, is subject to considerable experimental error.

- Frequently, experiments upon which QSAR analyses depend lack design in the strict sense of experimental design. Therefore the data collected may not reflect the complete property space. Consequently, many QSAR results cannot be used to confidently predict the most likely compounds of best activity.
- Various physicochemical parameters are known to be cross-correlated. Therefore only variables or their combinations that have little covariance should be used in a QSAR analysis; similar considerations apply when correlations are sought for different sets of biological data.

4. Conclusion

As shown in this chapter the flavonoids have been extensively studied for their applications and that is why many routes to flavonoids are described and possible. We only quoted herein the most interesting chemistry about the flavonoids, and our choice for a route to flavonoids has been focused on the easiness of the chemistry and its reproducibly in the chemical industry.

We wanted to use the QSAR to study and better understand the potential of flavonoids (or some classes of flavonoids). It is better to say that we investigated some SARs with no intention to assert new applications. The SARs were used as a directive guideline to help us in the choice of flavonoids as new cosmetic ingredients.

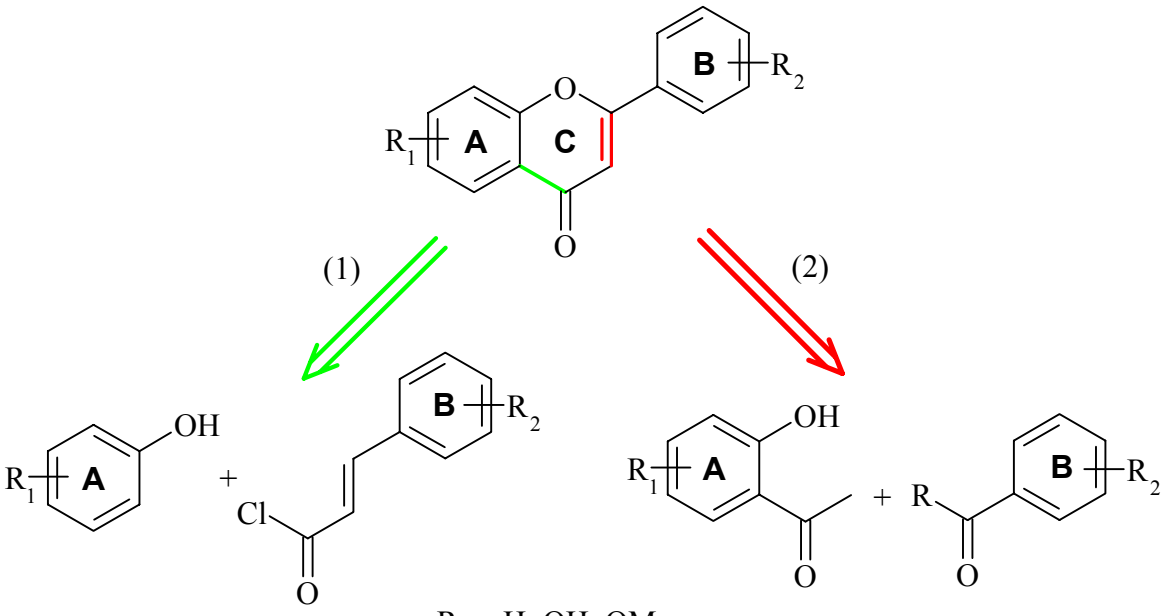
Chapter 2: The Syntheses of Flavonoids Precursors

1. Introduction

Flavonoids have been a topic of research for more than one century. Some scientists have been trying to get their extracts from different plants and different parts of the plants, to study their numerous interesting properties (see chapter one). Some started a course of some synthetically experiments to afford the flavonoids.

To access to flavonoids we distinguished two chemical routes (Scheme 21):

1. The substitution of a phenol with an α,β-unsaturated acyl chloride
2. The substitution of an acetophenone with a benzaldehyde, a benzoyl anhydride, or a benzoyl chloride.

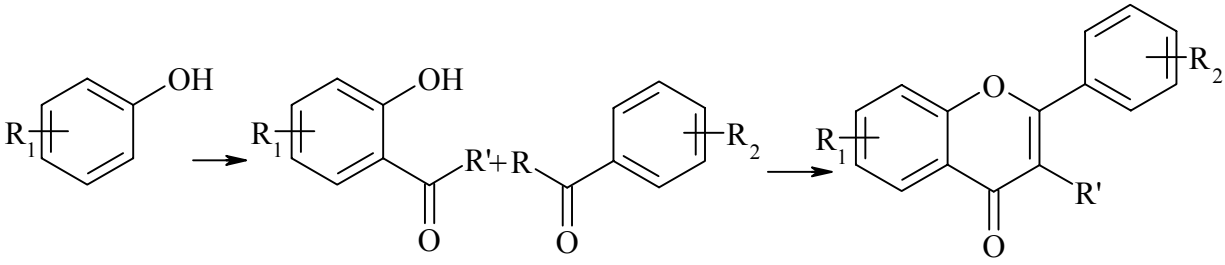


R_1 = H, OH, OMe
R_2 = H, OMe, Cl, NO_2
R = H, Cl

Scheme 21: The two main retrosyntheses of flavonoids

To make a choice between these two routes, we compared the availability of the reagents: phenols or acetophenones are largely available and not so expensive. Concerning the cinnamic acids or the benzaldehydes, a benzoyl chlorides or a benzoyl anhydrides, the second route offers more opportunities with a larger choice of reagents, and the costs of the full-step

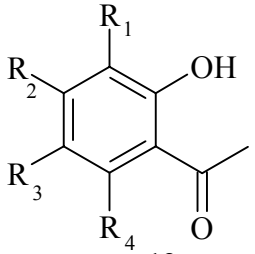
synthesis could be reduced in comparison with the cost of acetophenones and their syntheses. That is the reason why we focused our work on the second reaction: the condensation of an acetophenone with a benzaldehyde, a benzoyl chloride or a benzoyl anhydride (Scheme 22).



R_1 = H, OH, OMe
R_2 = H, OMe, Cl, NO_2
R = H, Cl
R' = Me, CH_2OMe, CH_2Ph

Scheme 22: Theoretical chemical synthesis to access the flavonoids

In order to reach the goal of the thesis (the Structure-Activity Relationships of flavones), we had to synthesise many flavonoids. The choice of acetophenones was determined by the examples given in nature and by the systematic study of the structure of flavonoids, that implies the variation of the position and of the number of the hydroxyl group on the phenyl rings. Thus, we restricted the combination on the position and the number of substituents. We decided to build flavonoids where the A-ring contains none, one or two hydroxyl groups.



12

12	**a**	**b**	**c**	**d**	**e**	**f**	**g**	**i**	**h**
R_1	H	OH	H	H	H	OH	H	H	H
R_2	H	H	OH	H	H	OH	-O CH_2 O-	OH	OH
R_3	H	H	H	OH	H	H		OH	H
R_4	H	H	H	H	OH	H	H	H	OH

Scheme 23 and Table 1: Selected hydroxyacetophenones.

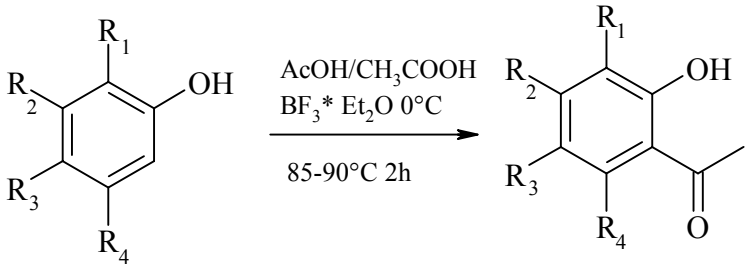
As the hydroxyl group in position 2 on the acetophenone is needed in the ring closure to form the C-ring of any flavonoids, it means that any chosen acetophenones should possess at least one (at the 2-position) and up to three hydroxyl groups. Thus, we selected the 2-hydroxyacetophenone (**12a**), the 2,3-, 2,4-, 2,5-, 2,6-dihydroxyacetophenones (**12b**, **12c**, **12d** and **12e** respectively) the 2,3,4-, 2,4,5- and 2,4,6-trihydroxyacetophenones (**12f**, **12i** and **12h** respectively) to be used (Scheme 23).

2. Syntheses of the acetophenones

2.1. Friedel-Crafts reaction

Many methods[107] are known to acylate a phenol. We wanted to reduce the costs of the general synthesis of flavonoids by comparing the price of commercial available acetophenones and the price of synthesized acetophenones in our laboratory. For this reason we decided to have a new step in the building block synthesis and to achieve major syntheses of acetophenone, BF_3 as complex in diethyl ether, seemed us to be the best catalyst for Friedel-Crafts-type reactions. The carboxylic anhydride / glacial acetic acid were chosen because they constitute a mild Friedel-Crafts acylating system with BF_3.

As indicated in Scheme 24, the reaction of phenol (**55a**), resorcinol (**55b**), hydroquinone (**55c**), pyrogallol (**55d**) phloroglucinol (**55e**) and sesamol (**55f**) in acetic acid and / or acetic anhydride, which was nearly saturated with boron trifluoride, gave easily the corresponding acetophenones in good yields. The reaction presented no difficulty, particularly in the work-up, because most of the complexed BF_3-acetophenones precipitate in the reaction mixture. When some complex are present in the final reaction mixture, the recrystallisation in methanol of the filtered complex, cleaves the bond between the oxygen and the boron, and regenerates the corresponding phenol.



55 **12**

55	R_1	R_2	R_3	R_4
a	H	H	H	H
b	H	OH	H	H
c	H	H	OH	H
d	OH	OH	H	H
e	H	OH	H	OH
f	H	-OCH_2O-		H

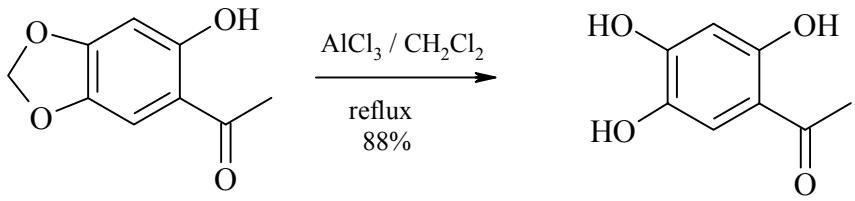
Table 2

12	R_1	R_2	R_3	R_4	Yield
a	H	H	H	H	92%
c	H	OH	H	H	91%
d	H	H	OH	H	87%
f	OH	OH	H	H	89%
h	H	OH	H	OH	85%
g	H	-OCH_2O-		H	86%

Table 3

Scheme 24: Acylation of polyphenols as precursors for the building blocks.

The cleavage of the methylendioxy group of compound (**12g**) was carried out following the method described by Hu *et al*[108] using aluminum tribromide but gave either degradation products or starting material. We opted for the cleavage method of Mauthner[109] with aluminum chloride. It cleaves the methylene-protecting group of **12g** to lead to the 2,4,5-trihydroxyacetophenone (**12i**) (Scheme 25) in 88%.



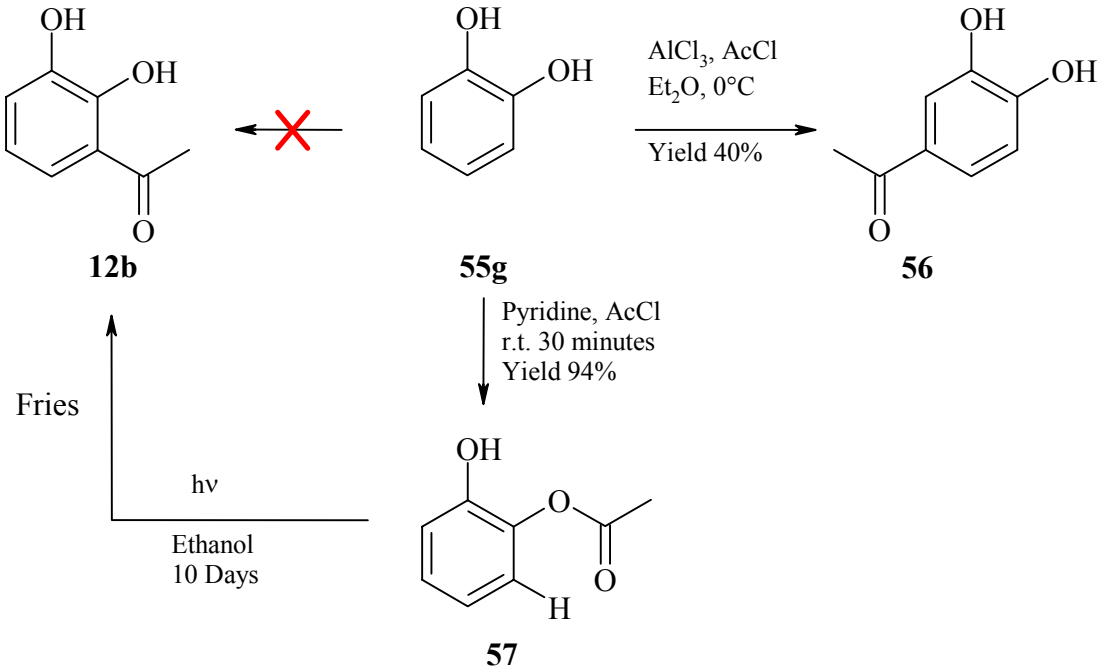
12g **12i**

Scheme 25: Deprotection of the phenolic hydroxy groups

2.2. Syntheses of the 2,3-dihydroxyacetophenone (12b)

It is chemically impossible to get the 2,3-dihydroxyacetophenone (**12b**) from an acylation of the catechol (**55g**), and a usual thermal induced Fries rearrangement in presence of catalyst gives the formation of *o*-hydroxy and *p*-hydroxyketones (in many cases both isomers are formed at the same time). Thus, we looked for an alternative route like a photo-induced Fries

rearrangement reaction that should avoid the formation of the *para* derivative (**56**) (Scheme 26).

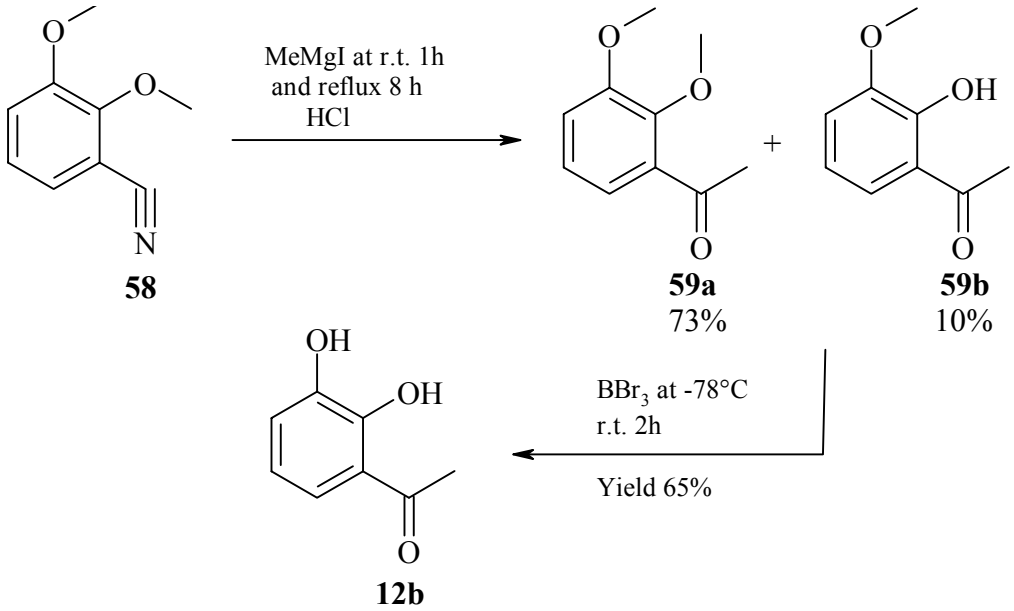


Scheme 26: Alternative route to the 2,3-Dihydroxyacetophenone

To apply the Fries rearrangement, we synthesised the acetic acid ester of benzene-1,2-diol (**57**), and purified it by distillation. The acetyl chloride is added to the catechol (**55g**) in presence of pyridine. A solution of acetic acid ester of benzene-1,2-diol (**57**) in ethanol was then irradiated in a photochemical reactor as described by Jefferson *et al.*[110] The colour of the solution changed after 24h and became darker. The reaction was followed by HPLC-control. After 10 days no formation of the desired acetophenone was observed.

An alternative method for the synthesis of the 2,3-dihydroxyacetophenone (**12b**) was the interaction of a Grignard reagent with the available benzonitrile (**58**), which gives directly the corresponding acetophenone (Scheme 27). The reaction of the 2,3-dimethoxybenzonitrile (**58**) with 1.5 equivalents of methyl magnesium iodide yields the 2,3-dimethoxyacetophenone (**59a**) as the major product and the 2-hydroxy-3-methoxyacetophenone (**59b**) as the minor product. Since the methoxyl group ortho to the acetyl is vinyl-analogous with methyl acetate it is possible that it could have suffered hydrolysis during the acid treatment to destroy the

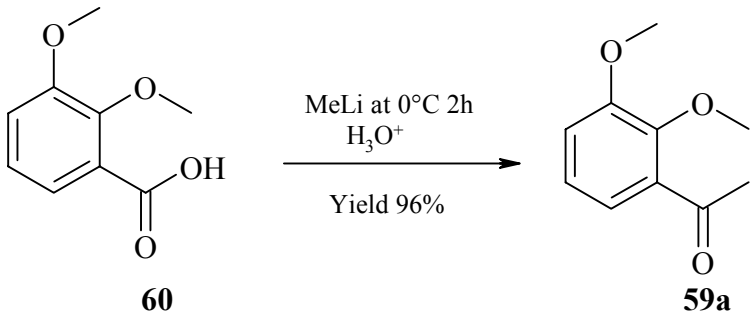
magnesium complex. However, Amstutz[111] showed that the splitting occurred during the reaction with the Grignard reagent. So instead of isolating both derivatives and demethylate



Scheme 27: Grignard reaction on 2,3-dimethoxybenzonitrile (**59a**)

each separately, we used the mixture of **59a** and **59b** in the demethylation reaction,[112] which have been carried out with boron tribromide (2.5 equivalents for each methyl to be splitted) at very low temperatures (-78°C), under nitrogen atmosphere and a vigorous stirring to afford the compound **12b** in 65% yield.

To avoid the formation of the phenol (**59b**) it might have been necessary to use more equivalents of Grignard reagent. We chose another route: the reaction of organometals and carboxylic acids for the synthesis of ketones to compare the easiness and cheapness of the synthesis of the 2,3-Dihydroxyacetophenone (**12b**) (Scheme 28).

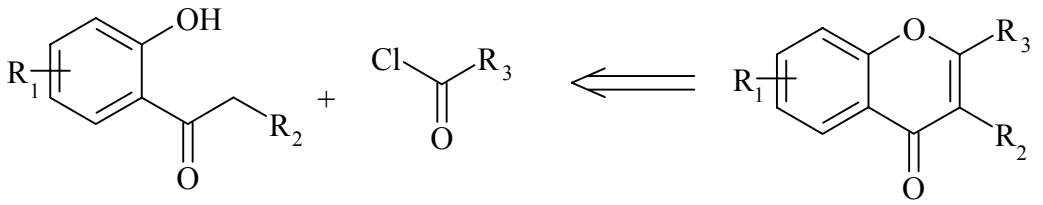


Scheme 28: Reaction of Methyl lithium

The addition of four equivalents of methyl lithium to the 2,3-dimethoxycarboxylic acid (**60**) at low temperatures and under inert atmosphere led to the 2,3-dimethoxyacetophenone (**59a**) that was deprotected with the same procedure as described before to afford the 2,3-dihydroxyacetophenone (**12b**). The organolithium reaction showed better yield (96%) than the Grignard reaction (83%) for the synthesis of the 2,3-dihydroxyacetophenone (**12b**).

3. Syntheses of α-substituted acetophenones

Flavonols and isoflavones can be synthesized using the same building block synthesis. For this reason, the acetophenones used are α-substituted (Scheme 29).



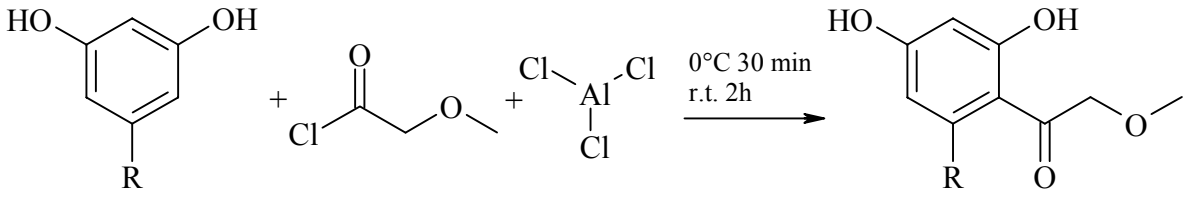
R_1 = H, OH
R_2 = OMe, Ph
R_3 = H, Ph

Flavonols:
R_1 = H,OH; R_2 = OMe; R_3 = Ph
Isoflavones:
R_1 = H,OH; R_2 = Ph; R_3 = H

Scheme 29: General retrosynthesis of other flavonoid classes

3.1. Precursors of flavonols

The reaction of resorcinol (**55b**), or phloroglucinol (**55e**) in presence of aluminum chloride and methoxyacetyl chloride (**61**) led to the corresponding α-methoxyacetophenones (**63a**) and (**63b**) (Scheme 30). Four equivalents of aluminium chloride were necessary to acetylate the resorcinol (**55b**) and only five equivalents to acetylate the phloroglucinol (**55e**).



55b R = H
55e R = OH

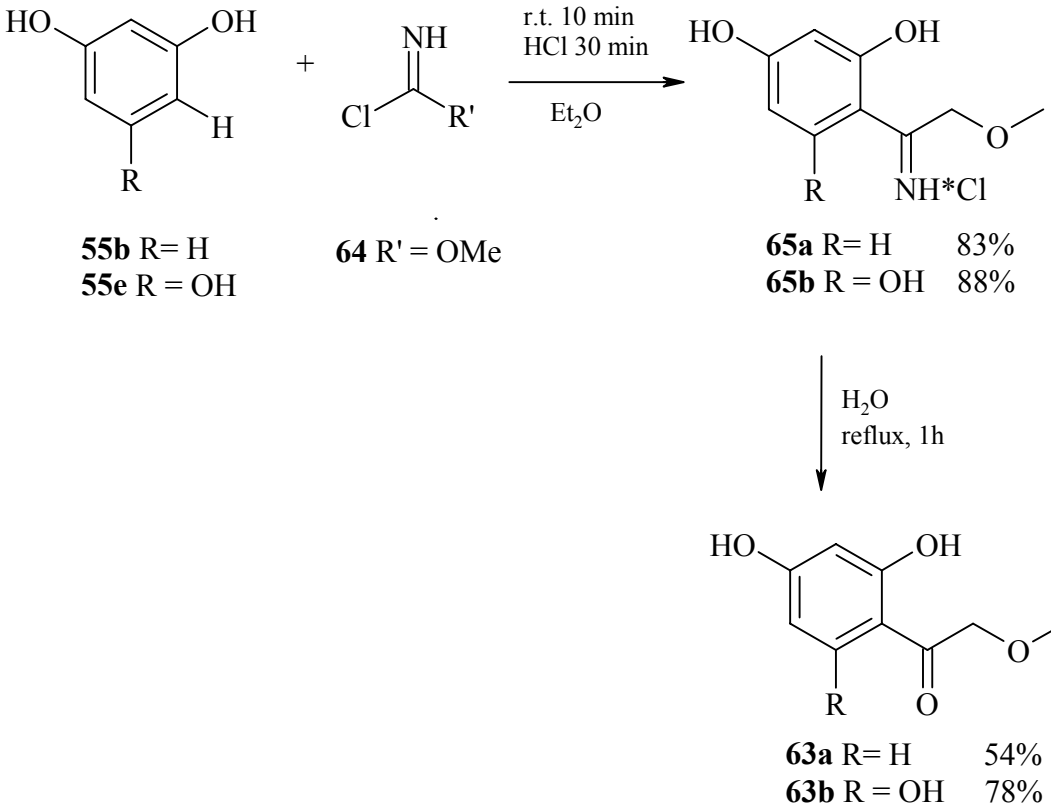
61

62

63a R = H, 98%
63b R = OH, 63%

Scheme 30: Acylation of phenol

An alternative synthesis of the α-substituted acetophenones is the Houben-Hoesch reaction. This reaction is more appropriated to the substituted phenols and proceeds most smoothly with polyvalent phenols whose hydroxyl groups are in *meta* position, such as resorcinol (**55b**) and phloroglucinol (**55e**). We carried out this synthesis with the phenols (**55b)** and (**55e**) in presence of HCl gas (Scheme 31).



55b R= H
55e R = OH

64 R' = OMe

65a R= H 83%
65b R = OH 88%

63a R= H 54%
63b R = OH 78%

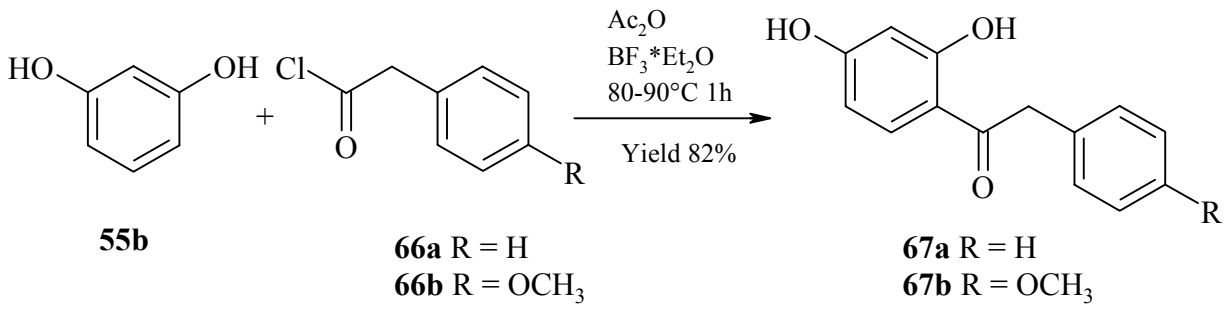
Scheme 31: The Houben-Hoesch reaction on phenolic compounds

Under these conditions the ketimine hydrochloride (**65**) is obtained, which can be easily converted into the corresponding acetophenone (**63**). The overall yields of the Houben-Hoesch reaction were 45% and 69% for resorcinol (**55b**) and phloroglucinol (**55e**) respectively.

3.2. Precursors of isoflavonoids

The flavonoid families include an important group of flavonoids called the isoflavones, which are the tautomers of the flavones. But their precursors are not the usual acetophenones. For this reason, we looked for the deoxybenzoins (an acetophenone α-substituted with an

aryl), which could lead to the isoflavones. We carried out the Friedel-Crafts reaction on resorcinol (**55b**) with the aryl acetyl chloride derivatives (**66a**) and (**66b**) (Scheme 32).



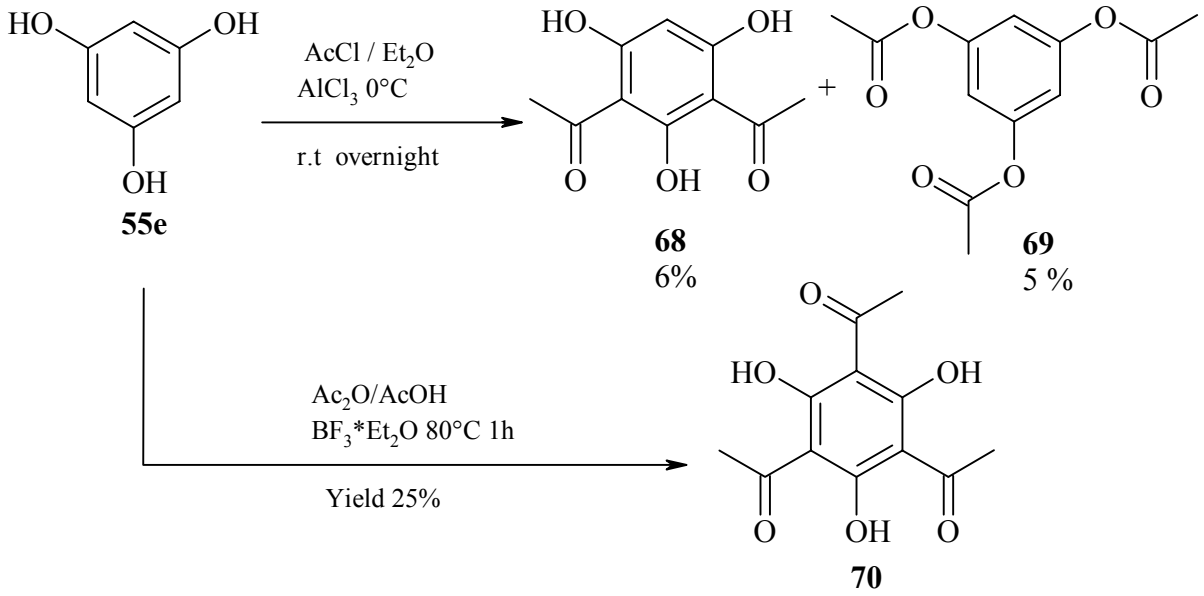
55b **66a** R = H, **66b** R = OCH_3 **67a** R = H, **67b** R = OCH_3

Scheme 32: Synthesis of Deoxybenzoin derivatives

Resorcinol (**55b**), in presence of BF_3, is stoichiometrically mixed to aryl acetyl chloride (**66a**) and (**66b**) at 85°C to yield crystalline pure deoxybenzoin derivatives (**67a**) and (**67b**) in 82%.

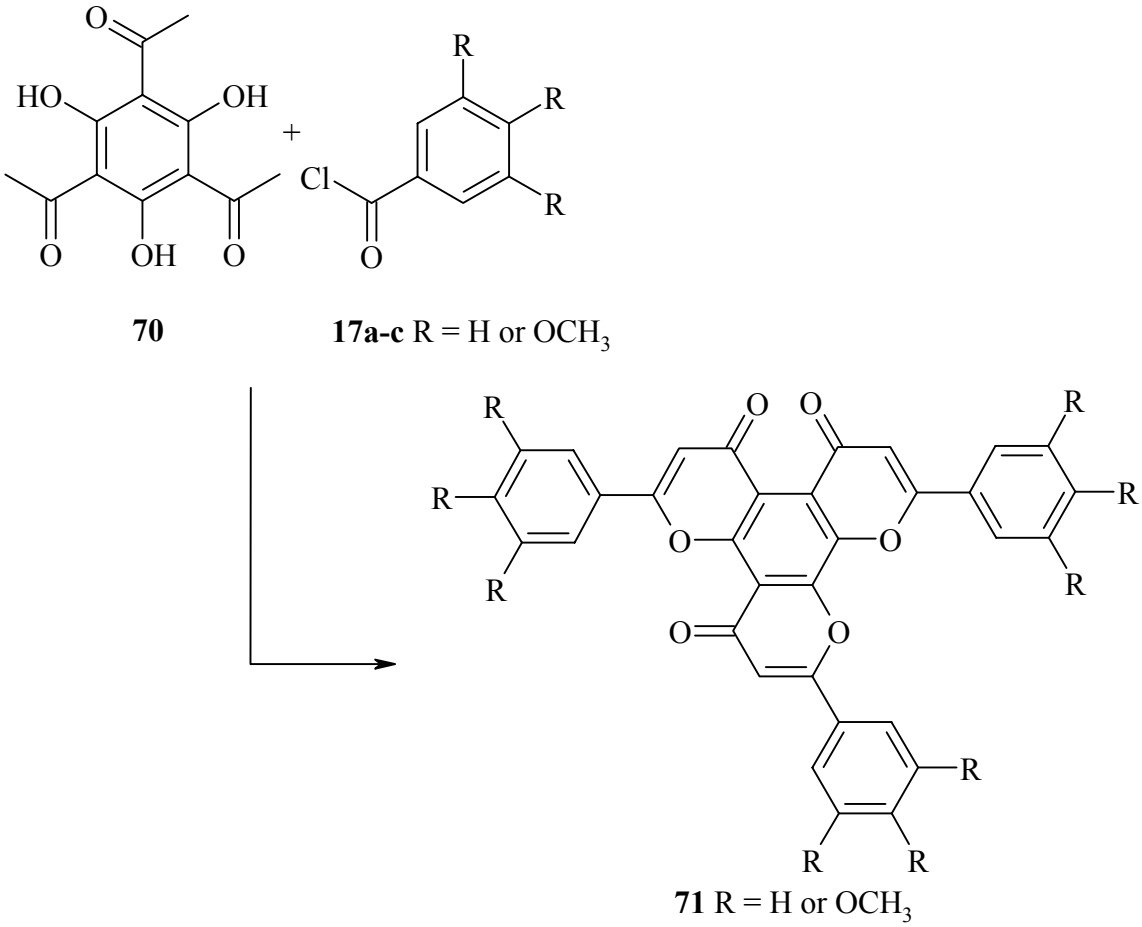
4. Polyacetylation of phenol

Usually polyacetylation of a phenolic substrate in a single step is rather rare as the substrate becomes less reactive after the introduction of an electron withdrawing acetyl group. We observed the formation of the 3-acetyl-2,4,6-trihydroxyacetophenone (**68**), accompanied by 1,3,5-triacetoxyphenyl (**69**) both as second products, when we carried out the reaction with aluminum chloride catalyst (Scheme 33). With four equivalents of aluminum chloride and one equivalent of acetyl chloride, the diacetylation of phloroglucinol and the substitution of each hydroxyl group of the phenol with the acetyl chloride are observed and lead to the diacetyl (**68**) and the triester (**69**) instead of forming the 2,4,6-trihydroxyacetophenone (**12e**) in these proportions. The 3,5-diacetyl-2,4,6-trihydroxyacetophenone (**70**) was obtained in relative higher yields (25%) with boron trifluoride (Scheme 33). By the triacetylation of phloroglucinol, we first tested the reaction with 3.4 equivalents of boron trifluoride and it led to the 3,5-diacetyl-2,4,6-trihydroxyacetophenone (**70**). When we reduced the boron equivalent to 1.3, it afforded the 1,3,5-triacetoxyphenyl (**69**) in 81%. The boron must complex every hydroxyl groups at the same time in order to polyacetylate the phenol.



Scheme 33: Polyacetylation of phloroglucinol

To design new flavones, we used the polyacetophenone (**70**) in order to obtain polyflavonoids fused on the A-ring (Scheme 34). Unfortunately the reaction was uncompleted and it was not possible to obtain the desired fused triflavones (**71**).



Scheme 34: Representation of fused-triflavones

5. Conclusion

The syntheses of the desired acetophenones were possible through different kind of reactions but the choice of the reactions and its reagents have been guided by the availability of the reagents and not by the cheapness or easiness of the reactions. Phenols are not ideal substrates for the Friedel-Crafts reaction, because of the competition between the hydroxyl group and the aromatic ring to react with the acetyl chloride. In our case most of the phenols were symmetrical molecules and led to only one product. We have tested several catalysts to get the best yield and we observed that boron trifluoride is the most appropriated catalyst to carry out Friedel-Crafts reaction on phenol when the price of an available acetophenone is to expensive. The reaction is easy, quick and sometimes needs no phases extraction, which can be appreciable for the industrial scale-up.

For the synthesis of flavonoids (Chapter 3), we used at the beginning our synthesized acetophenones and for the improvement of the reactions we took all commercially available acetophenones.

Chapter 3: New synthetic routes to flavonoids

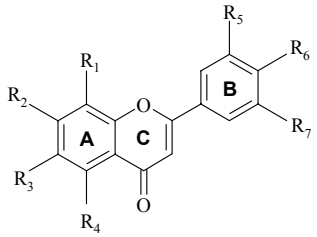
1. Introduction

By developing a synthesis for flavonoids we wanted to design flavones with different substitution patterns in a model library. According to Iinuma and Mizuno,[113] 38627 flavones and flavonols substituted (on A-, B- and C-ring) with hydroxyl and/or methoxyl groups are possible theoretically. As discussed in chapter II, we selected the structure of each flavone in comparison with the flavones found in nature which present some biological activities. We thought that the flavonols could be synthesized following the same approach, if we considered them as flavones with a hydroxyl group at the 3-position. This position can be introduced in the skeleton of the flavones by modifying the structure of the acetophenone on the acetyl part.

2. Syntheses of flavones

Although a number of methods are available for the synthesis of flavones, they are not ideal for the preparation of ring-A hydroxylated flavones. The main disadvantage of those methods is that it requires multiple operations in which several protecting groups (benzoate or MOM groups) had to first be introduced and then removed after rearrangement and cyclodehydration steps. The results are often partial deprotection of the phenolic hydroxyl groups, which lowers the overall yield and complicates the isolation procedure.

In view of these considerations, we looked for syntheses which avoid the formation of the derivatized intermediates of phenolic hydroxyl groups as esters or ethers. And in the same approach, we looked for easy and cheap chemistry in order to transfer it in a possible industrial scale-up.



Compd	R_1	R_2	R_3	R_4	R_5	R_6	R_7	Method*	Yield, %†
24	H	H	H	H	H	H	H	A	85
72	OH	H	H	H	H	H	H	-	-
73	H	OH	H	H	H	H	H	A	12
74	H	H	OH	H	H	H	H	A	25
75	H	H	H	OH	H	H	H	A	49
76	OH	OH	H	H	H	H	H	A	39
77	H	OH	OH	H	H	H	H	B	37
78	H	OH	H	OH	H	H	H	C	47
79	OH	OH	OH	H	H	H	H	-	-
80	H	H	H	H	H	OCH_3	H	A	48
81	OH	H	H	H	H	OCH_3	H	-	-
82	H	OH	H	H	H	OCH_3	H	B	61
83	H	H	OH	H	H	OCH_3	H	A	7
84	H	H	H	OH	H	OCH_3	H	B	57
85	OH	OH	H	H	H	OCH_3	H	A	7
86	H	OH	OH	H	H	OCH_3	H	-	-
87	H	OH	H	OH	H	OCH_3	H	C	55
88	H	H	H	H	OCH_3	OCH_3	H	A	94
89	OH	H	H	H	OCH_3	OCH_3	H	-	-
90	H	OH	H	H	OCH_3	OCH_3	H	A	51
91	H	H	OH	H	OCH_3	OCH_3	H	A	47
92	H	H	H	OH	OCH_3	OCH_3	H	B	89
93	OH	OH	H	H	OCH_3	OCH_3	H	A	20
94	H	OH	OH	H	OCH_3	OCH_3	H	A	24
95	H	OH	H	OH	OCH_3	OCH_3	H	B	29
96	H	H	H	H	OCH_3	OCH_3	OCH_3	C	23
97	OH	H	H	H	OCH_3	OCH_3	OCH_3	-	-
98	H	OH	H	H	OCH_3	OCH_3	OCH_3	A	14
99	H	H	OH	H	OCH_3	OCH_3	OCH_3	A	13
100	H	H	H	OH	OCH_3	OCH_3	OCH_3	C	36
101	OH	OH	H	H	OCH_3	OCH_3	OCH_3	A	10
102	H	OH	OH	H	OCH_3	OCH_3	OCH_3	A	18
103	H	OH	H	OH	OCH_3	OCH_3	OCH_3	C	45
104	H	H	H	H	H	OH	H	-	99
105	OH	H	H	H	H	OH	H	-	-
106	H	OH	H	H	H	OH	H	-	99
107	H	H	OH	H	H	OH	H	-	76
108	H	H	H	OH	H	OH	H	-	82
109	OH	OH	H	H	H	OH	H	-	93
110	H	OH	OH	H	H	OH	H	-	-
111	H	OH	H	OH	H	OH	H	-	90
112	H	H	H	H	OH	OH	H	-	76
113	OH	H	H	H	OH	OH	H	-	-
114	H	OH	H	H	OH	OH	H	-	94
115	H	H	OH	H	OH	OH	H	-	86
116	OH	H	H	OH	OH	OH	H	-	96
117	OH	OH	H	H	OH	OH	H	-	85
118	H	OH	OH	H	OH	OH	H	-	-
119	H	OH	H	OH	OH	OH	H	-	77
120	H	H	H	H	OH	OH	OH	-	81
121	OH	H	H	H	OH	OH	OH	-	-
122	H	OH	H	H	OH	OH	OH	-	-
123	H	H	OH	H	OH	OH	OH	-	-
124	H	H	H	OH	OH	OH	OH	-	100
125	OH	OH	H	H	OH	OH	OH	-	89
126	H	OH	OH	H	OH	OH	OH	-	37
127	H	OH	H	OH	OH	OH	OH	-	17
128	H	H	H	OH	H	Cl	H	B	90
129	H	H	H	OH	H	NO_2	H	B	27
130	H	H	H	OH	H	NH_2	H	-	99
131	H		-OCH_2O-	H		-OCH_2O-	H	B	22

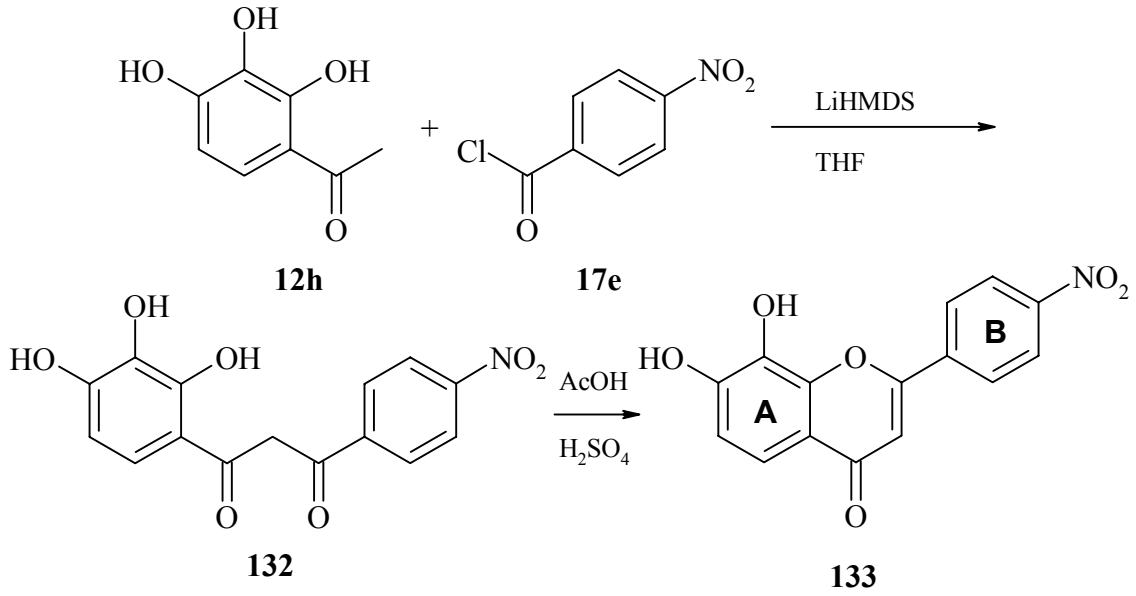
Table 4: Our model library of designed and synthesized flavones **24-131**

* A: synthesis with LiHMDS; B: synthesis with LiOH; C: synthesis BK-VK with LiOH.

† The synthesis was carried out in order to isolate the compound and not to get the best yield of the method.

2.1. Building Block Approach

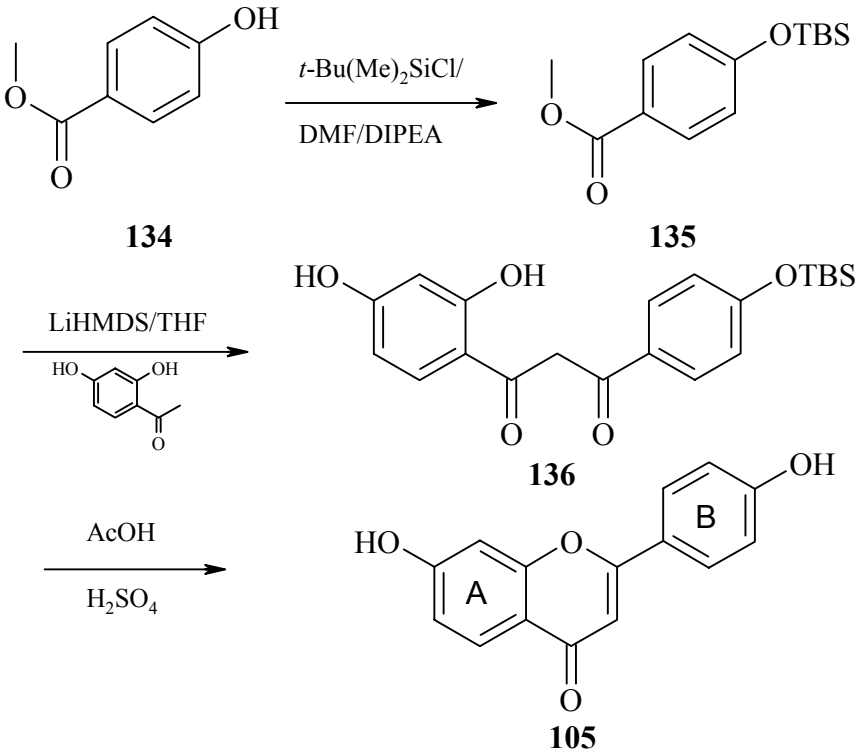
Few years ago, Cushmann and Nagarathnam[114] devised a method for A-ring hydroxylated flavone synthesis (called **Method A**) that was short, avoided the formation of *O*-aroylated intermediates, and provided the desired products in high yield (89-96%) without side product. The key step in this process is the generation of lithium polyanions from the polyhydroxylated acetophenones (**12**) using enough equivalent lithium bis(trimethyl)silyl amide (LiHMDS) to ensure the generation of the lithium enolates from the acetyl groups. Lithium polyanions, as intermediates, offer the advantage to avoid a multistep protection-deprotection procedure and the formation *O*-aroylated intermediates. In Scheme 35, the treatment of the lithium polyanions of the 2,3,4-trihydroxyacetophenone (**12h**) with one equivalent of 4-nitrobenzoyl chloride (**17e**) afforded the 1-(2,3,4-trihydroxyphenyl)-3-(4-nitrophenyl)-1,3-propanediones (**132**). It was immediately cyclized to the corresponding 7,8-dihydroxy-4′-nitroflavone (**133**) upon heating at 95-100°C in glacial acetic acid containing 0.5% sulphuric acid.



Scheme 35: Cushman and Nagarathnam facile synthesis of A-ring hydroxylated flavones

Cushmann and Nagarathnam documented the extension of this A-ring hydroxylated flavones synthesis. In combination with *tert*-butyldimethylsilyl protection[115] of the B-ring phenolic hydroxyls, it led to the preparation of a variety of flavones bearing hydroxyl groups

on both the A and B rings (**Method A′**). The desired polyhydroxylated flavones were produced in high yields (76-96%) (Scheme 36).

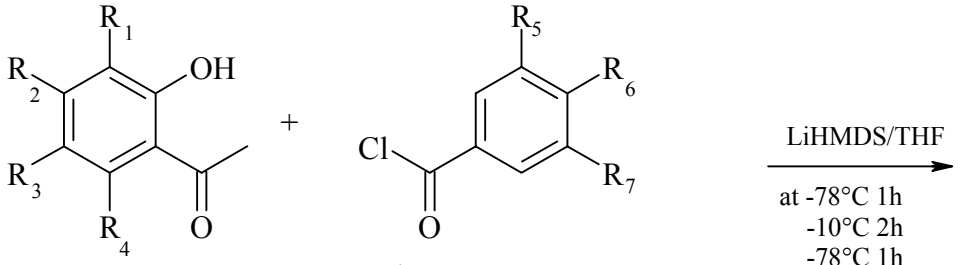


Scheme 36: variation of Cushman and Nagarathman method with *O*-sililated benzoates.

We opted for the methodology of the A-ring hydroxylated flavones (**Method A**), because **Method A′** implied the synthesis of all ether of aroyl and sometimes the preparation of both ester and ether precursors and only leads to one kind of substituted flavones: the polyhydroxylated flavones. We wanted a method, that would allow us to understand the role of the substitution pattern in relation to their biological activity. When selecting an aroyl chloride with a methoxyl, or nitro substitution, we were able to obtain some other flavones derivatives *via* short and easy chemical transformations.

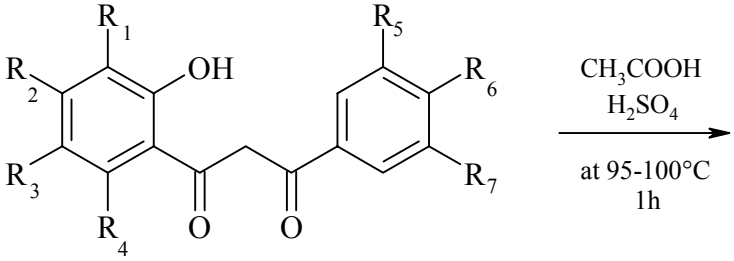
The mono-, di-, tri- and tetrahydroxyacetophenones (**12**) were employed as starting material, and transformed into lithium polyanions with enough equivalents* of the lithium bis(trimethylsilyl)amide. In the critical step of the process they were condensed with the aroyl chloride acids (**17**) to afford the 1-aryl-3-(2-hydroxyphenyl)-1,3-propanediones (**132**). Those intermediates were immediately subjected to cyclodehydration with 0.5% sulphuric acid in

* one equivalent of LiHMDS is required for each hydroxyl group, one other for the keto group and one in excess

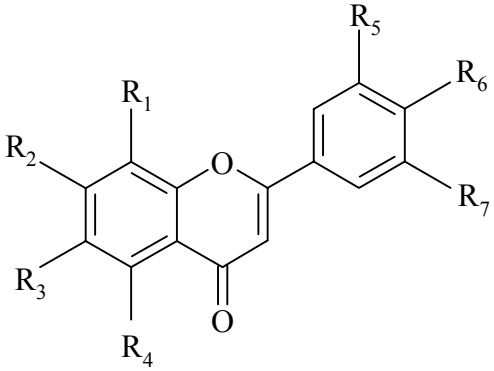


12a R_1,R_2,R_3,R_4=H
12b R_1=OH; R_2,R_3, R_4= H
12c R_2=OH; R_1,R_3,R_4=H
12d R_3=OH; R_1,R_2, R_4=H
12e R_4=OH; R_1, R_2,R_3=H
12f R_1,R_2=OH; R_3,R_4=H
12g R_2,R_3=OH; R_1,R_4=H
12h R_2,R_4=OH; R_1,R_3= H
12i R_2,R_3=OCH_2O; R_1,R_4=H

17a R_5,R_6,R_7 =H
17b R_6=OCH_3, R_5,R_7 = H
17c R_5,R_6=OCH_3, R_7 =H
17d R_5,R_6, R_7=OCH_3
17e R_6=Cl,R_1, R_5,R_7 =H
17f R_6=NO_2, R_5,R_7 = H
17g R_5,R_6=OCH_2O; R_7=H



132a R_1=R_2=R_3=R_4=R_5=R_6=R_7=H
132c R_1=R_3=R_4=R_5=R_6=R_7=H, R_2=OH
132d R_1=R_2=R_4=R_5=R_6=R_7=H, R_3=OH
132e R_1=R_2=R_3=R_5=R_6=R_7=H, R_4=OH
132f R_3=R_4=R_5=R_6=R_7=H, R_1=R_2=OH
132g R_1=R_4=R_5=R_6=R_7=H, R_2=R_3=OH
132h R_1=R_3=R_5=R_6=R_7=H, R_2=R_4=OH
132i R_1=R_2=R_3=R_4=R_5=R_7=H, R_6=OMe
132k R_1=R_3=R_4=R_5=R_7=H, R_2=OH, R_6=OMe
132l R_1=R_2=R_4=R_5=R_7=H, R_3=OH, R_6=OMe
132m R_1=R_2=R_3=R_5=R_7=H, R_4=OH, R_6=OMe
132n R_1=R_3=R_5=R_7=H, R_1=R_2=OH, R_6=OMe
132o R_1=R_4=R_5=R_7=H, R_2=R_3=OH, R_6=OMe
132p R_1=R_4=R_5=R_7=H, R_2=R_4=OH,R_6=OMe
132q R_1=R_2=R_3=R_4=R_7=H, R_5=R_6=OMe
132s R_1=R_3=R_4=R_7=H, R_2=OH, R_5=R_6=OMe
132t R_1=R_2=R_4=R_7=H, R_3=OH, R_5=R_6=OMe
132u R_1=R_2=R_3=R_7=H, R_4=OH, R_5=R_6=OMe
132v R_3=R_4=R_7=H, R_1=R_2=OH, R_5=R_6=OMe
132w R_3=R_4=R_7=H, R_2,R_3=OH, R_5=R_6=OMe
132x R_1=R_4=R_5=H, R_2=R_4=OH, R_6=R_7=OMe
132y R_1=R_2=R_3=R_4=H, R_5=R_6=R_7=OMe
132a´ R_1=R_3=R_4=H, R_2=OH, R_5=R_6=R_7=OMe
132b´ R_1=R_2=R_4=H, R_3=OH, R_5=R_6=R_7=OMe
132c´ R_1=R_2=R_3=H, R_4=OH, R_5=R_6=R_7=OMe
132d´ R_1=R_3=H, R_1=R_2=OH, R_5=R_6=R_7=OMe
132e´ R_3=R_4=H, R_1=R_2=OH, R_5=R_6=R_7=OMe
132f´ R_1=R_4=H, R_2=R_3=OH, R_5=R_6=R_7=OMe
132g' R_1=R_2=R_3=R_5=R_7=H, R_4=OH, R_6=Cl
132h' R_1=R_2=R_3=R_5=R_7=H, R_4=OH, R_6=NO_2

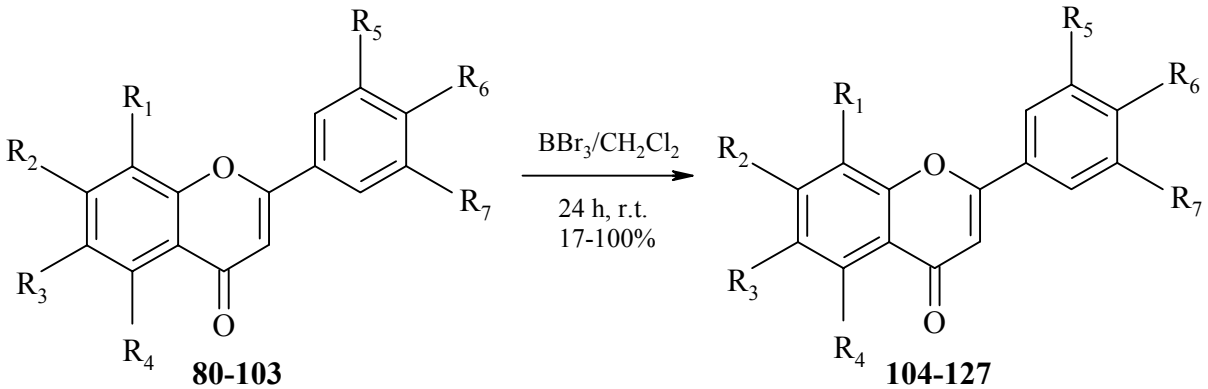


Compounds **24**-**103**, **128**, **129** and **131** listed in Table 4

Scheme 37: Synthesis of the model library of flavones

glacial acetic acid to afford the desired flavones with yields ranging from 7 to 94 % (listed in Table 4, Scheme 37).

In order to get a maximum of flavones derivatives, we used the methoxylated flavones **80-103** as starting material (see Table 4, compounds with R_5, R_6 and R_7 = H, OMe) in a demethylation process. The total cleavage of their methyl ethers in presence of boron tribromide led to the corresponding polyhydroxylated flavones (**104-127**) in yields ranging from 17 to 100% (Scheme 38).

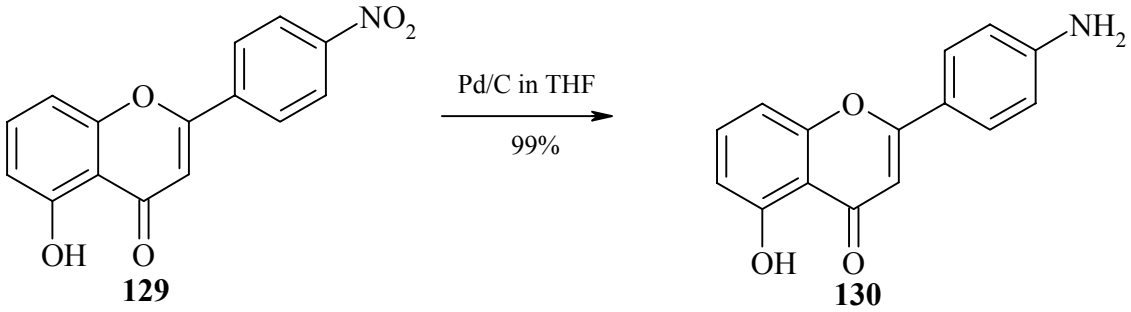


80-103
R_1,R_2,R_3,R_4=H,OH
R_5,R_6,R_7=H,OMe

104-127
R_1,R_2,R_3,R_4,R_5,R_6,R_7= H,OH

Scheme 38: Total demethylation of methoxylated flavones **104** to **127**

Among all the substitution patterns of flavones, we speculated that an amino group could be very interesting as such or for further chemical transformation. Thus, we carried out the hydrogenation of the 5-hydroxy-4′-nitroflavone (**129**) in presence of palladium and under hydrogen atmosphere to yield the 4′-amino-5-hydroxyflavone (**130**) in 99% (Scheme 39).



129 **130**

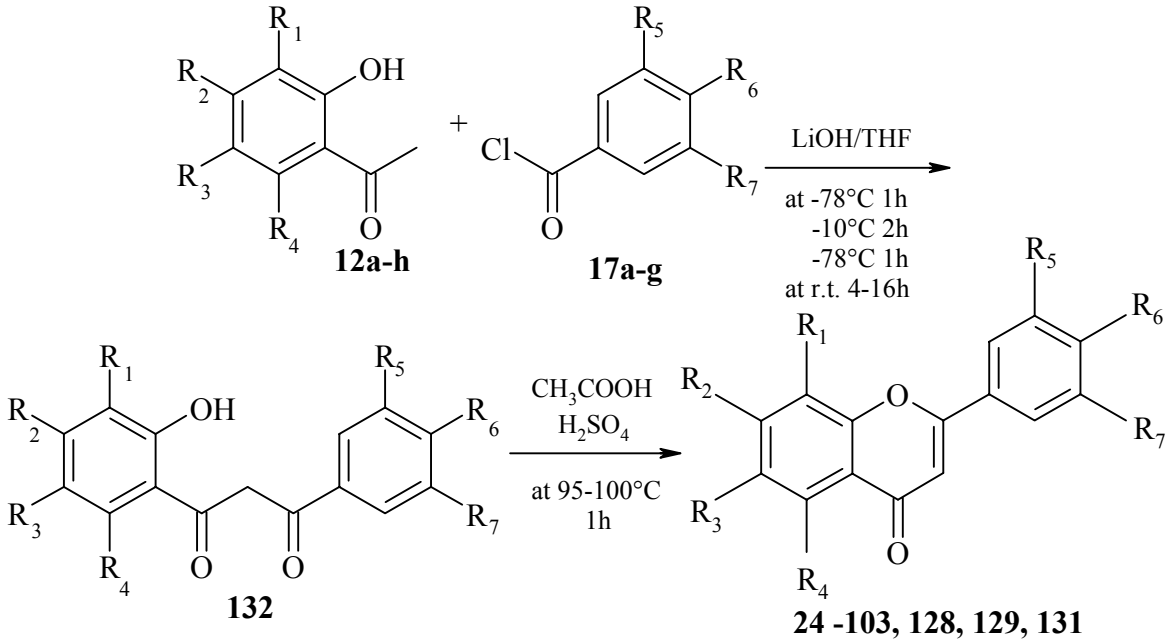
Scheme 39: Hydrogenation of the nitroflavone

However, we realised that the cost of the lithium bis(trimethylsilyl)amide and its way to handle (the solution is inflammable, sensitive to air and must be picked up with a one-way

syringe under argon, special equipments are needed in pilot), were important drawbacks and we started to look for another method or another base which could be used under similar conditions.

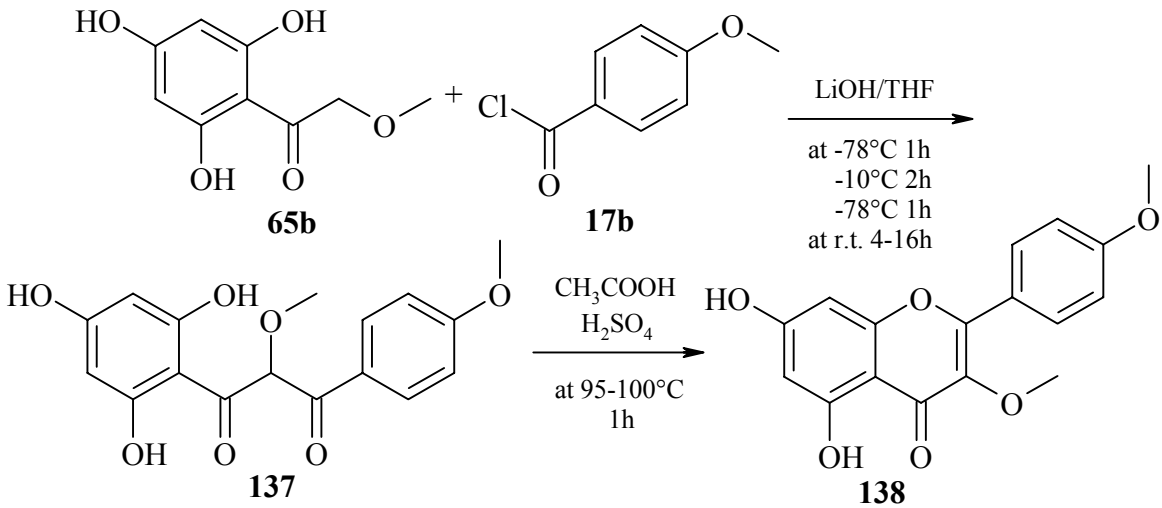
2.2. A new procedure with Lithium hydroxide

Unexpectedly, we found[116] that flavones (Scheme 40) could be synthesised in a one-step procedure (following the **Method A**) using lithium hydroxide (called **Method B**).



Scheme 40: New procedure with LiOH to obtain flavones

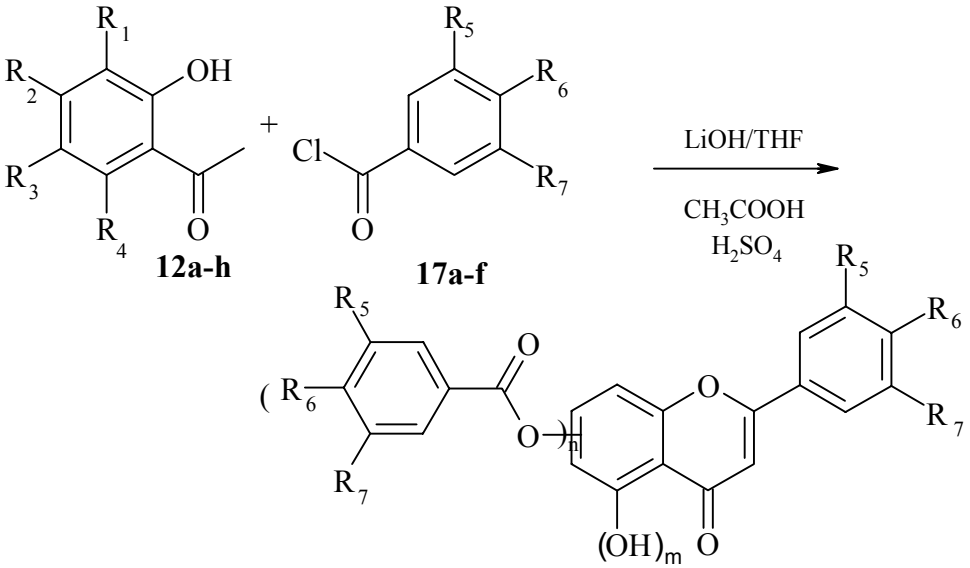
We managed to extend this new method on flavonols (Scheme 41) and isolated the Kaempherol 3,4′-dimethyl ether (**138**).



Scheme 41: New procedure with LiOH to obtain flavonols.

Our new conditions present less equivalents* of the lithium base, so that the excess used in **method A** is no longer necessary. We can also notice that LiOH is not soluble in THF, forming then a homogeneous gel or paste with the acetophenone and the lithium base. Price of the base and the heterogeneous conditions delivered a great advantage for this reaction.

However, we noticed that the lithium polyanions formed with LiOH were more sensitive to the variation of the reaction conditions than those formed with LiHMDS. So if the temperature did not decrease enough or was not maintained at - 78°C during the addition of the aroyl chloride on the lithium polyanions, the aroyl chloride could react on any hydroxyl groups to afford the corresponding aroyl substituted flavones as ester (Scheme 42).



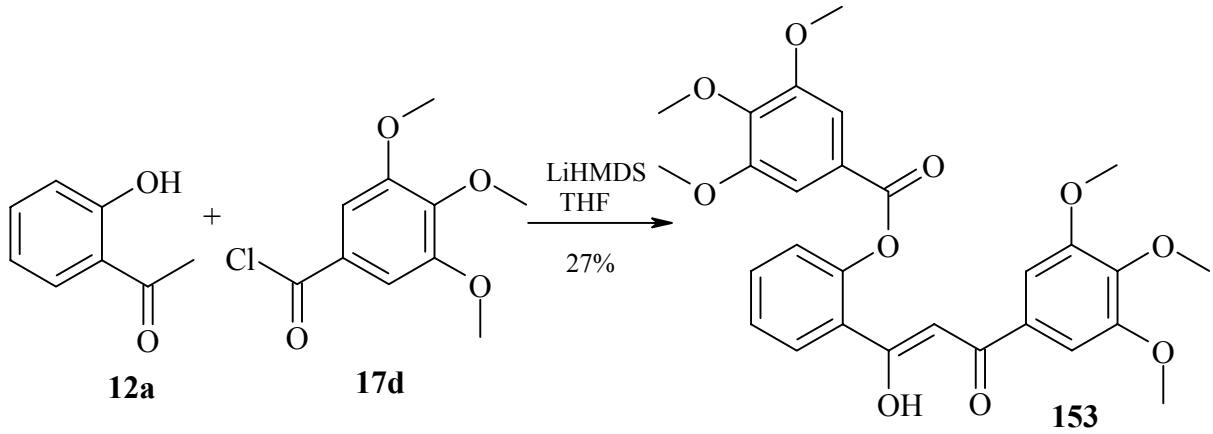
139-152 R_5, R_6, R_7 = H,OMe; n = 1,2; m = 0,1

Compd N°	Corresponding flavone	Position of the aroyl	Benzoyl derivative	Yield %
139	73	7	Benzoic acid	4
140	74	6	Benzoic acid	33
141	82	7	4′-Methoxybenzoic acid	4
142	83	6	4′-Methoxybenzoic acid	11
143	87	7	4′-Methoxybenzoic acid	16
144	90	7	3′,4′-Dimethoxybenzoic acid	4
145	91	6	3′,4′-Dimethoxybenzoic acid	55
146	95	7	3′,4′-Dimethoxybenzoic acid	6
147	94	6+7	3′,4′-Dimethoxybenzoic acid	8
148	98	7	3′,4′,5′-Trimethoxybenzoic acid	9
149	99	6	3′,4′,5′-Trimethoxybenzoic acid	13
150	103	7	3′,4′,5′-Trimethoxybenzoic acid	11
151	101	7	3′,4′,5′-Trimethoxybenzoic acid	14
152	-	7	Benzo[1,3]dioxole-5-carboxylic acid	-

Scheme 42 and Table 5: Observation of the formation of flavone esters

* Only hydroxyl groups and the ketone are taken in account to calculate the number of equivalent

The formation of the ester did not depend on the type of aroyl chloride used in the reaction. We noticed that the substitutions of the 7-, and 6-position of the flavones were often isolated and the 5-position was never subjected to the formation of an ester. It can be explained by the presence of a hydrogen bond between the 5-hydroxyl group and the 4-keto group. All the esters appeared as side products and were separated from the reaction mixture during the crystallization process in a second or third fraction. Among the side products isolated during all syntheses, we also observed the formation of an ester of diketone (**153**) as main product (Scheme 43).



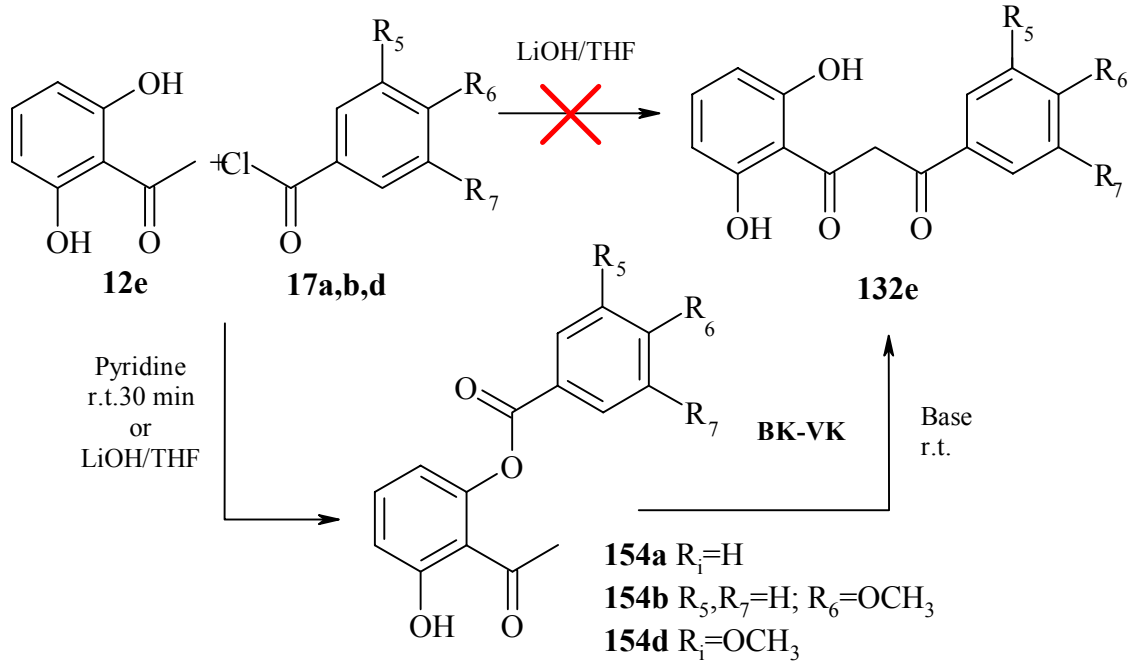
Scheme 43: Formation of an ester of diketone (**153**)

2.3. A modified Baker-Venkataraman rearrangement for the synthesis of flavonoids

The formation of aroyl ester derivatives of flavones happened more often during the improvement of the conditions of the reaction, we decided to understand the origin of their formation and try to avoid it through different conditions using the lithium base (LiOH).

To understand the formation of the aroyl derivatives, we isolated each intermediates. We first carried out the synthesis of the easier molecule the 5-hydroxyflavone, which should not be esterificated. We did not observe the formation of the corresponding expected 1,3-diketone (**132e**), but we characterized the 6-benzoyloxy-2-hydroxyacetophenone (**154**). This compound can also be obtained by mixing stoechiometrically the 2,6-dihydroxyacetophenone (**12e**), the benzoyl chloride (**17a**) and pyridine (Scheme 44).

It was observed that a stoichiometrically mixture of the 2,6-dihydroxyacetophenone (**12e**) with lithium hydroxide at room temperature with the benzoyl chloride (**17a**) led to the derivative (**154**).

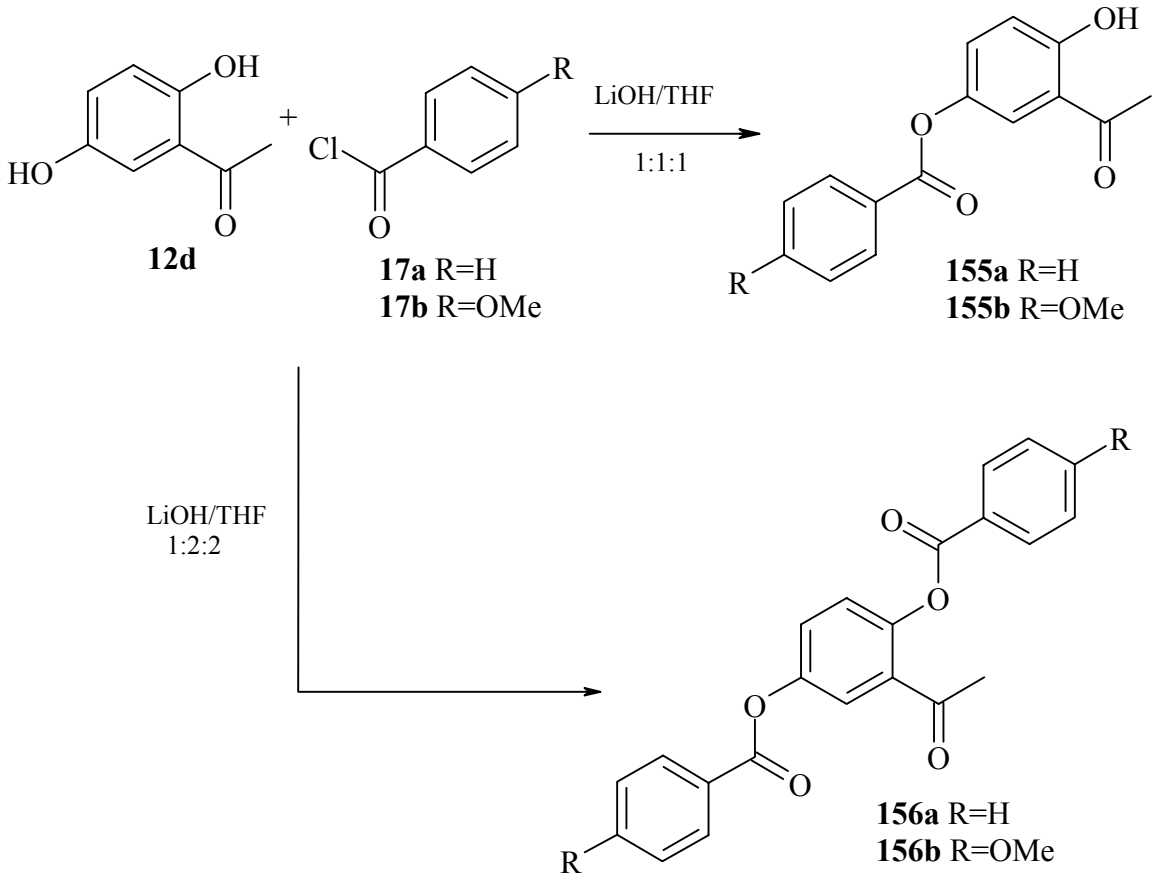


Scheme 44: Analysis of the first step of the synthesis, the formation of 1,3-diketone

By forming an aroyloxyacetophenone, it was easy to use the Baker-Venkataraman pathway, in which a 2-aroylacetophenone undergoes a base catalysed rearrangement to the corresponding 1,3-diketone, to afford the intermediate **132e** (Scheme 44).

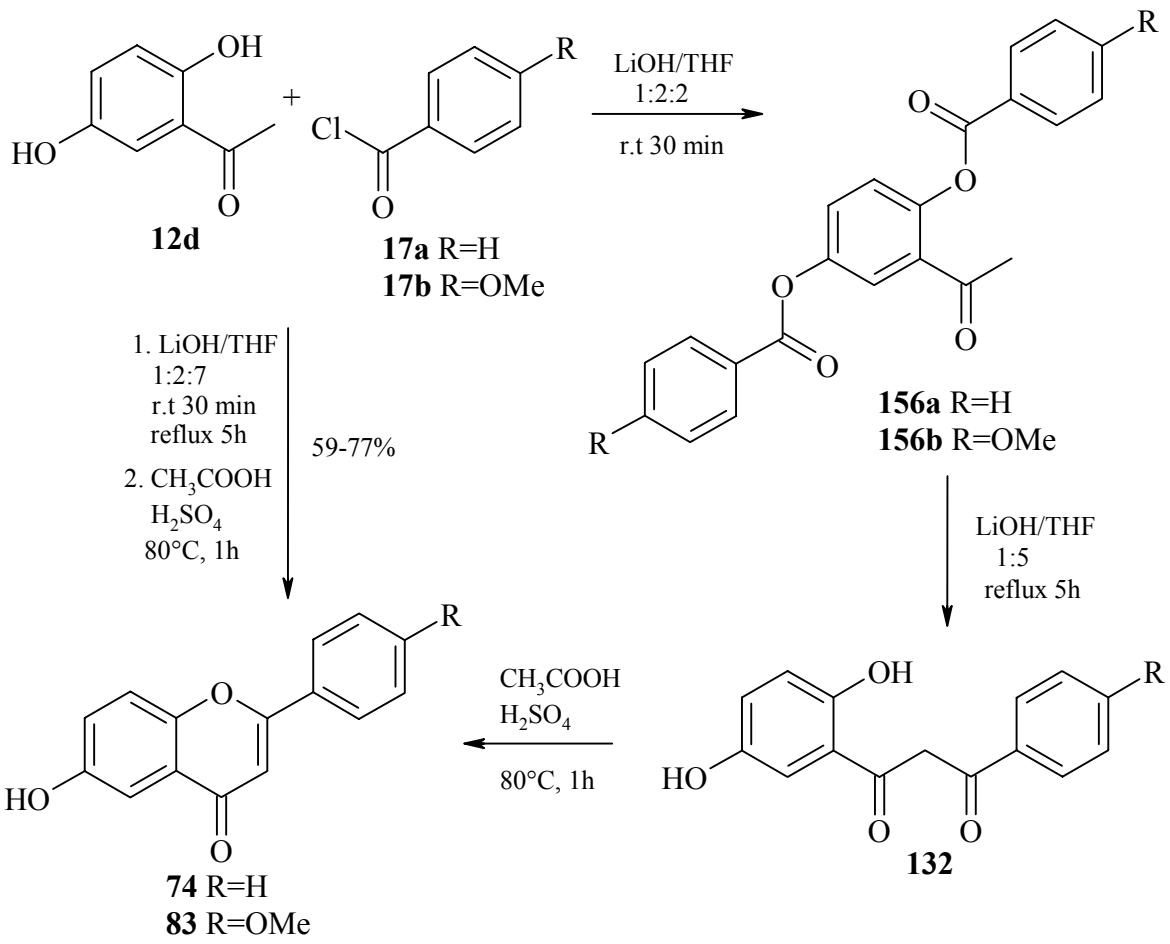
We replaced the 2,6-dihydroxyacetophenone (**12e**) with the 2,5-dihydroxyacetophenone (**12d**) and mixed it stoichiometrically (one equivalent of each starting materials, symbolized by 1:1:1) with lithium hydroxide and the corresponding aroyl chloride at room temperature, we isolated the isomer 5-aroyloxy-2-hydroxyacetophenone (**155**). At this point of the reaction, we suspected that the aroyl chloride would first react on the most distant hydroxyl group from the acetyl of the acetophenone, because of the presence of an labile hydrogen bond between the 2-hydroxyl group and the keto group. In this case, it was impossible to apply the BK-VK, so the number of equivalents of lithium hydroxide and benzoyl chloride was raised to two

(symbolised by 1:2:2) to obtain a complete substitution of the hydroxyl groups of the acetophenone (Scheme 45).



Scheme 45: Aroyl substitution of the 2,5-dihydroxyacetophenone (12d)

The Baker-Venkataraman rearrangement has always been carried out with either bases such as Na, NaH, LiH, or solvents such as DMF, DMSO. We assumed that LiOH was basic enough to take place in such a reaction and we carried out the BK-VK rearrangement with LiOH. An extensive study revealed that five equivalents of the base (for the BK-VK rearrangement and the cleavage of the aroyl groups) and five hours of reaction time were required for optimal yields of the diketones (**132**). After recrystallisation overall yield of the steps transforming the 2-aroyl derivatives (**156**) into flavones ranged from 59 to 77% yield (Scheme 46).

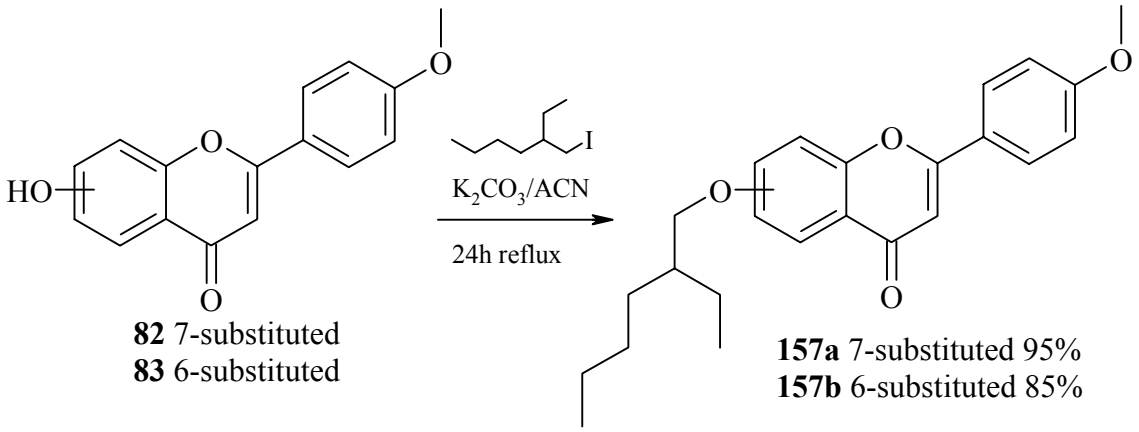


Scheme 46: a new approach via the BK-VK rearrangement with LiOH

Finally, the hydroxyl groups were deprotected by saponification of the benzoate flavone derivatives. The compounds were heated at 60°C two hours in a hydroalcoholic solution (10/90) with 5% of sodium hydroxide.

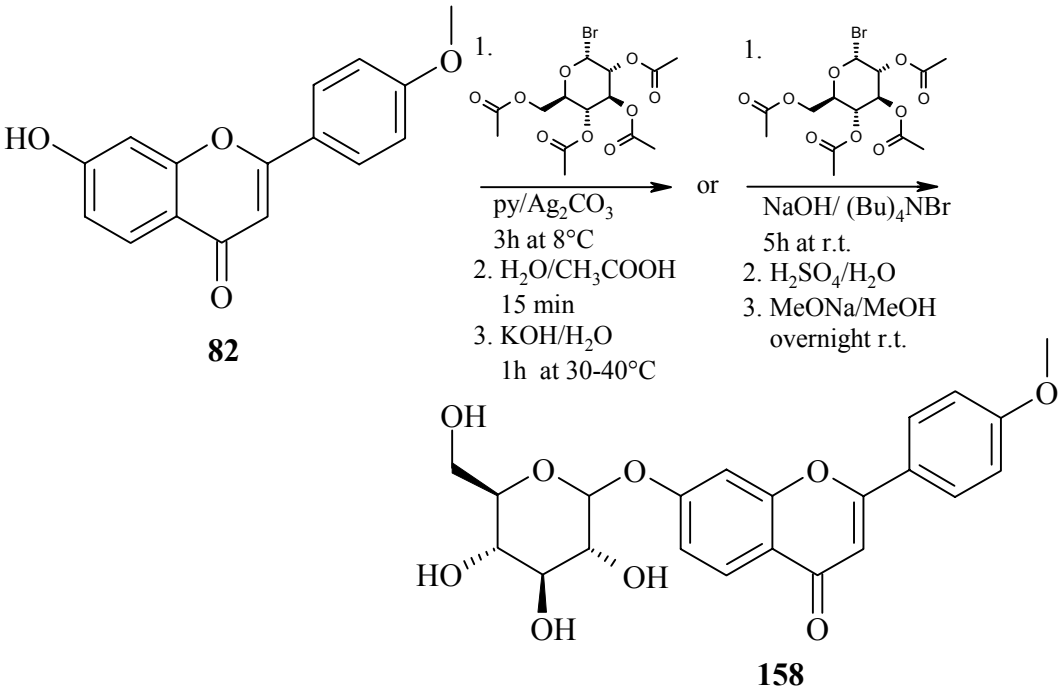
3. Cosmetic solubilization

Cosmetics formulation takes place in two mediums: hydrophilic or hydrophobic medium. The polyhydroxylated flavonoids showed a weak solubility potential in both media. By functionalising one hydroxyl group with an alkyl chain or a glycosyl substituent it was possible to enhance their solubility respectively in the lipidic or aqueous formulation. The 7-hydroxy-4′-methoxyflavone (**82**) or 6-hydroxy-4′-methoxyflavone (**83**) was mixed with the 2-ethylhexyliodid and potassium carbonate in acetonitrile to afford the 7- or 6-ethylhexyloxy-4′-methoxyflavone (**157**), respectively in 95% and 85% yield (Scheme 47).



Scheme 47: Synthesis of lipophilic flavonoids

The synthesis of the glucosyl-flavone (**158**) was carried out following two methods: by dissolving the 7-hydroxy-4′-methoxyflavone (**82**) with D-(+)-alpha-acetobromide glucose and silver carbonate in pyridine. The addition of potassium hydroxide on the intermediate product led to the cleavage of acetyl group and yielded the 7-*O*-glucosyl-4′-methoxyflavone (**158**) in 20%. Or by dissolving the 7-hydroxy-4′-methoxyflavone (**82**) in sodium hydroxide and adding D-(+)-alpha-acetobromide with ammonium tetrabutylbromide in dichloromethane. Thus, the intermediate product was deprotected in presence of sodium methylate in methanol to afford the 7-*O*-glucosyl-4′-methoxyflavone (**158**) in 64% yield.



Scheme 48: Synthesis of hydrophilic flavonoid (**158**)

4. Conclusion

Polyhydroxylated flavonoids used to be difficult to synthesise, because of the formation of *O*-aroyl substitutions, or obtained with low yields because of several step syntheses (including protection and deprotection of the hydroxyl group). We modified the Cushman and Nagarathman synthesis with lithium hydroxide and developed it to be also used in industrial scale-up considering the easiness and cheapness of the synthesis. We extended this new method to the synthesis of flavonols by isolating the Kaempherol 3,4′-dimethyl ether (**138**).

This synthetic approach constitutes a simple and good yielding way to synthesize diverse flavonoids (flavones and flavonols) using available and various starting materials (like acetophenones and aroyl chloride). This method can be applied as a routine synthesis or as an automatized synthesis in the field of parallel synthesis.

PART II

Chapter 4: Structure-^{13}C Nuclear Magnetic Resonance Assignment Relationship

1. Introduction

NMR spectroscopy, since the nearly simultaneous initial reports by Bloch[117] and Purcell[118] in 1946 has emerged as one of the most powerful and indispensable techniques available for the study of molecular structure and dynamics in chemistry. It permits, for example, the quantitative analysis of mixtures, determination of molecular structure, characterisation of interactions between molecules and measurement of reaction rates in the steady state, all simultaneously on a single sample.

The sensitivity of commercially available NMR spectrometers has increased dramatically for the last 50 years, due to the development of super conducting magnets and greatly improved electronics. The sensitivity of these machines to carbon-13 is, unfortunately, nowhere now as great. This is not surprising since the natural abundance of carbon-13 is only 1.1 per cent and, with a magnetic moment only a quarter that of hydrogen, it is intrinsically 62.5 times harder to detect. The combination of these two factors results in an overall sensitivity that is 5700 times less than for protons. In spite of this, however, with the current NMR instrumentation, a reasonable spectrum can be obtained from about one milligram of flavonoid after overnight acquisition.

The first report dealing with the ^{13}C NMR Study of flavonoids compounds[119] appeared in 1974. Since then large numbers of publications reporting ^{13}C NMR data on flavonoids have been published.[120,121] It is not intended to discuss here the instrumental or theoretical aspects of this technique.

2. Structure-^{13}C NMR Assignment Relationship

We compared here the chemical shifts of the carbon atoms present in the different synthesized flavonoids. Each atom has a electron density around its nucleus, which can vary with the neighbourhood of each nucleus, with the result that each chemically distinct carbon

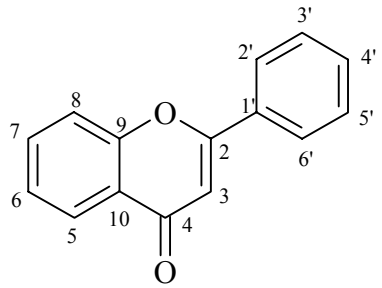
atom in a structure will come into a resonance at a slightly different frequency form all the others. The electrons affect the microenvironment, because their movement creates a magnetic field. The factors affecting the chemical shift are, in our case, most of the time intramolecular factors.

The inductive effect: a high electron density shields a nucleus, and causes resonance to occur at relatively high field and such nuclei are said to be *shielded* by the electron. Likewise, a low electron density causes resonance to occur at relatively low field, and the nucleus is said to be *deshielded*. Then the electropositive elements shift the signal upfield, and the electronegative elements shift the signals downfield, because they donate and withdraw electrons, respectively.

Anisotropy of chemical bonds: Chemical bonds are also region of high electron density that can set up magnetic fields. These fields are stronger in one direction than another, and this effect of the field on the chemical shift of nearby nuclei is dependent upon the orientation of the nucleus in question with respect to the bond.

2.1. Flavones

In the scope of our systematic research on the flavonoids, we describe here the ^{13}C NMR data of synthesised compounds, which are partially hydroxylated and methoxylated (Table 6) and the numbering system is mentioned on Scheme 49. The chemical shift of different carbon atoms of these compounds are gathered in Table 7 and Table 8.



Scheme 49: Chemical structure of flavones

Cpd. n°	Position							Cpd. n°	Position						
	5	6	7	8	3′	4′	5′		5	6	7	8	3′	4′	5′
24	H	H	H	H	H	H	H	**101**	H	H	OH	OH	OCH_3	OCH_3	OCH_3
72	H	H	H	OH	H	H	H	**102**	H	OH	OH	H	OCH_3	OCH_3	OCH_3
73	H	H	OH	H	H	H	H	**103**	OH	H	OH	H	OCH_3	OCH_3	OCH_3
74	H	OH	H	H	H	H	H	**104**	H	H	H	H	H	OH	H
75	OH	H	H	H	H	H	H	**105**	H	H	H	OH	H	OH	H
76	H	H	OH	OH	H	H	H	**106**	H	H	OH	H	H	OH	H
77	H	OH	OH	H	H	H	H	**107**	H	OH	H	H	H	OH	H
78	OH	H	OH	H	H	H	H	**108**	OH	H	H	H	H	OH	H
79	OH	OH	OH	H	H	H	H	**109**	H	H	OH	OH	H	OH	H
80	H	H	H	H	H	OCH_3	H	**110**	H	OH	OH	H	H	OH	H
81	H	H	H	OH	H	OCH_3	H	**111**	OH	H	OH	H	H	OH	H
82	H	H	OH	H	H	OCH_3	H	**112**	H	H	H	H	OH	OH	H
83	H	OH	H	H	H	OCH_3	H	**113**	H	H	H	OH	OH	OH	H
84	OH	H	H	H	H	OCH_3	H	**114**	H	H	OH	H	OH	OH	H
85	H	H	OH	OH	H	OCH_3	H	**115**	H	OH	H	H	OH	OH	H
86	H	OH	OH	H	H	OCH_3	H	**116**	OH	H	H	H	OH	OH	H
87	OH	H	OH	H	H	OCH_3	H	**117**	H	H	OH	OH	OH	OH	H
88	H	H	H	H	OCH_3	OCH_3	H	**118**	H	OH	OH	H	OH	OH	H
89	H	H	H	OH	OCH_3	OCH_3	H	**119**	OH	H	OH	H	OH	OH	H
90	H	H	OH	H	OCH_3	OCH_3	H	**120**	H	H	H	H	OH	OH	OH
91	H	OH	H	H	OCH_3	OCH_3	H	**121**	H	H	H	OH	OH	OH	OH
92	OH	H	H	H	OCH_3	OCH_3	H	**122**	H	H	OH	H	OH	OH	OH
93	H	H	OH	OH	OCH_3	OCH_3	H	**123**	H	OH	H	H	OH	OH	OH
94	H	OH	OH	H	OCH_3	OCH_3	H	**124**	OH	H	H	H	OH	OH	OH
95	OH	H	OH	H	OCH_3	OCH_3	H	**125**	H	H	OH	OH	OH	OH	OH
96	H	H	H	H	OCH_3	OCH_3	OCH_3	**126**	H	OH	OH	H	OH	OH	OH
97	H	H	H	OH	OCH_3	OCH_3	OCH_3	**127**	OH	H	OH	H	OH	OH	OH
98	H	H	OH	H	OCH_3	OCH_3	OCH_3	**128**	OH	H	H	H	H	Cl	H
99	H	OH	H	H	OCH_3	OCH_3	OCH_3	**130**	OH	H	H	H	H	NH_2	H
100	OH	H	H	H	OCH_3	OCH_3	OCH_3	**131**	H	OCH_2O		H	OCH_2O		H

Table 6: Synthetic studied flavonoids

We discussed in the following paragraphs the influence of a structural change on the skeleton of the flavonoids on the assignment of the carbon shifts. For each weak change, we considered a type of molecule and its corresponding derivatives, which did not possess the structural change. For example, for the introduction of on hydroxyl group at the 7-position, we selected: to compare to:

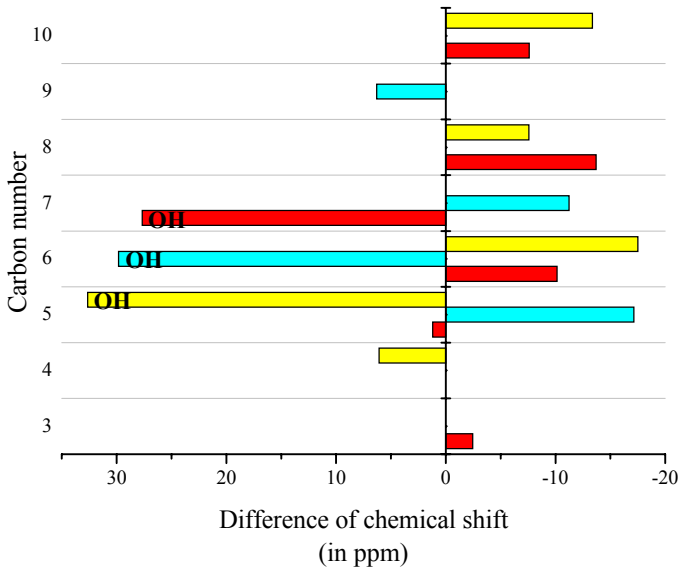
- 7-hydroxyflavone ⇔ Flavone
- 7-hydroxy-4′-methoxyflavone ⇔ 4′-methoxyflavone
- 7-hydroxy-3′,4′-dimethoxyflavone ⇔ 3′,4′-dimethoxyflavone
- 7-hydroxy-3′,4′,5′-trimethoxyflavone ⇔ 3′,4′,5′-trimethoxyflavone …

Then we compared each chemical shift of each carbon and calculated the difference of chemical shift between the unsubstituted derivatives and the substituted derivatives. As we had several differences of shifts, we calculated using statistics methods the average of the difference and the standard deviation, which are given in the text as + or – 0.00±0.00 ppm. In some case the standard deviation is dramatically important and is due to the fact that some of the chemical shifts values are quoted from some publications with indecisiveness about the assignment of the chemical shifts.

2.1.1. A- and C-rings

2.1.1.1. Introduction of one hydroxyl group

If we consider the flavones **24**, **80**, **88**, **96**, **104**, **112** and **120**, and introduce one hydroxyl group at any position on the A-ring, we can notice some perturbations due to the presence of the hydroxyl group on the carbon where it is linked and its neighbours. The case of one hydroxyl group at the 7-position (flavones **73**, **82**, **90**, **98**, **106**, **114** and **122)**, causes a perturbation essentially on the carbon atoms: 3 (–2.46±0.62 ppm), 5 (+1.20±0.23 ppm), 6 (–10.15±0.24 ppm), 7 (+27.66±0.58 ppm), 8 (–13.71±1.01 ppm), and 10 (–7.59±0.32 ppm). The presence of one OH group at the 6-position (flavones **74**, **83**, **91**, **99**, **107** and **115**) causes a perturbation only on the carbon atoms: 5 (–17.15±0.24 ppm), 6 (+29.83±0.17 ppm), 7 (–11.23±0.07 ppm) and 9 (–6.30±0.07 ppm), while the OH group at the 5-position (flavones **75**, **84**, **92**, **100**, **108** and **116**), affects more the carbon atoms: 4 (+6.08±0.09 ppm), 5 (+32.64±2.20 ppm), 6 (–17.51±0.35 ppm), 8 (–7.57±0.05 ppm), and 10 (–



Comp. n°	Position of the carbon														
	2	3	4	5	6	7	8	9	10	1′	2′	3′	4′	5′	6′
24	162.41	106.84	176.97	124.67	125.36	134.13	118.39	155.55	123.32	131.02	126.22	128.97	131.67	128.97	126.22
73	161.82	102.46	176.32	126.45	114.98	162.70	106.53	157.40	116.06	131.20	126.06	128.96	131.42	128.96	126.06
74	162.10	105.88	176.93	107.52	154.87	123.00	119.71	149.34	124.22	131.34	126.13	128.96	131.47	128.96	126.13
75	164.07	105.63	183.18	159.82	107.48*	135.92	110.95*	155.88	110.11	130.52	126.57	129.12	132.26	129.12	126.57
76	161.76	106.03	176.90	115.16	114.07	150.56	131.39	146.68	116.95	133.10	126.30	128.96	131.46	128.96	126.30
77	161.33	103.10	176.16	107.18	144.61	152.32	105.85	150.76	116.03	131.46	125.92	128.96	131.21	128.96	125.92
78	163.03*	105.08	181.73	161.43	98.96	164.36*	94.03	157.37	103.93	130.67	126.25	128.97	131.81	128.97	126.25
79	162.85	104.43	182.05	146.97	129.29	153.59	93.98	149.81	104.26	130.92	126.21	129.00	131.70	129.00	126.21
80	162.65	105.42	176.85	125.34	124.70	134.06	118.38	155.59	123.22	123.28	128.18	114.55	162.14	114.55	128.18
82	162.00	102.48	176.24	126.41	114.83	162.58	105.07	157.37	116.07	123.42	127.93	114.48	162.87	114.48	127.93
83	162.23	104.44	176.78	105.52	154.73	122.77	119.61	149.24	123.44	124.14	127.97	114.46	161.93	114.46	127.97
84	164.22	104.04	182.99	159.87	107.45*	135.72	110.86*	155.86	109.92	122.62	128.61	114.57	162.56	114.57	128.61
85	164.84	104.57	176.75	115.04	113.83	150.37	132.98	146.52	116.86	123.16	128.12	114.38	161.84	114.38	128.12
87	163.13*	103.40	181.67	161.35	98.78	164.11*	93.94	157.22	103.60	122.70	128.18	114.44	162.18	114.44	128.18
88	162.44	105.71	176.93	125.31	124.67	134.00	118.43	155.59	123.30	123.30	109.46	149.02	151.96	111.72	119.87
90	162.03	102.57	176.32	126.39	114.82	162.58	105.39	157.40	116.08	123.35	109.33	148.99	151.70	111.70	119.39
91	162.20	104.72	176.81	107.42	154.68	122.70	119.62	149.21	123.50	124.11	109.23	148.90	151.68	111.59	119.69
92	164.13	104.23	182.99	155.73	107.39*	135.61	110.77*	159.76	109.88	122.56	109.43	148.93	152.31	111.57	120.26
93	161.84	104.89	176.78	115.10	113.84	150.47	132.90	146.63	116.86	123.77	109.59	148.92	151.69	111.67	119.90
94	162.63	104.93	176.86	119.66	144.29	157.59	107.44	146.17	122.86	127.98	114.24	148.80	151.83	116.80	120.01
95	163.24*	103.77	181.75	161.35	98.78	164.15*	94.03	157.05	103.77	122.82	109.35	148.93	152.05	111.60	119.96
96	162.27	103.92	177.04	125.38	124.62	134.05	118.56	155.54	123.17	126.32	106.73	153.14	140.44	153.14	106.73
98	162.64	102.72	176.40	126.42	107.30	161.76	106.52	157.43	114.76	126.16	103.53	153.21	140.44	153.21	103.53
99	161.98	103.99	176.96	107.44	154.82	122.88	119.87	149.30	124.15	126.67	105.82	153.21	140.44	153.21	105.82
100	163.91	104.42	183.19	155.84	107.64*	135.00	110.92*	159.79	110.03	125.72	105.49	153.25	141.10	153.25	105.49
101	161.59	104.27	176.85	116.80	114.03	150.74	132.84	146.71	115.19	126.83	106.05	153.13	140.34	153.13	106.05
102	161.21	103.29	176.18	107.46	144.52	152.19	105.82	150.72	115.99	126.90	103.66	153.14	140.05	153.14	103.66
103	162.90*	104.92	181.80	161.30	98.87	164.28*	94.22	157.30	103.73	125.29	103.97	153.13	140.58	153.13	103.97

Table 7: chemical shift of ^{13}C NMR of partially methoxylated flavonoids in ppm in DMSO-*d* ^{6}

* Values may be switched

Comp. n°	Position of the carbon														
	2	3	4	5	6	7	8	9	10	1′	2′	3′	4′	5′	6′
104[122a]	163.1	104.9	176.9	125.3	124.8	133.9	118.3	155.4	123.4	121.7	128.4	116.0	161.0	116.0	128.4
106	162.33	104.37	176.19	126.36	114.67	162.43	102.37	157.27	116.01	121.68	128.02	115.78	160.58	115.78	128.02
107	162.59	103.80	176.70	107.45	154.62	122.62	119.53	149.14	124.10	121.74	128.10	115.81	160.68	115.81	128.10
108	163.54	103.24	182.85	161.40	107.22*	135.51	110.72*	155.67	109.82	120.85	128.67	115.91	159.78	115.91	128.67
109	162.15	103.88	176.69	114.94	113.68	150.22	132.94	146.42	116.80	121.93	128.20	115.71	160.55	115.71	128.20
111	164.01*	102.72	181.64	161.35	98.73	163.61*	93.85	157.19	103.60	121.07	128.35	115.84	161.05	115.84	128.35
112	164.19	104.80	176.75	133.92	125.19	135.67	118.74	155.51	123.38	121.90	113.34	145.68	149.40	115.95	124.67
114	162.50	102.26	176.16	126.39	114.68	162.45	104.39	157.24	115.99	122.05	113.06	145.48	149.04	115.88	118.44
115	162.76	103.84	176.63	107.47	154.61	122.63	118.55	149.14	124.10	122.10	113.16	145.61	149.14	115.88	119.46
116	164.71	103.29	182.73	159.79	107.13*	135.50	110.71*	155.66	109.81	121.10	113.48	145.70	149.96	115.95	119.26
117	162.34	103.82	176.64	114.87	113.33	150.09	133.04	146.61	116.87	122.30	113.59	145.62	149.00	115.80	118.61
119	163.78*	102.75	181.56	161.37	98.73	164.06*	93.74	157.18	103.57	121.37	113.24	145.64	149.61	115.90	118.90
120	163.62	102.09	176.67	124.26	135.72	137.90	117.95	154.25	124.63	121.06	107.28	145.67	137.90	145.67	107.28
121[122b]	164.2	103.2	181.6	161.6	99.0	164.2	93.9	157.5	104.0	120.8	106.0	146.5	137.9	146.5	106.0
122[122c]	163.1	104.8	176.6	126.8	115.1	162.8	102.5	157.6	116.3	121.4	105.7	146.5	137.5	146.5	105.7
125	162.60	103.85	176.48	114.82	113.55	150.02	133.13	146.68	116.96	121.31	105.56	146.16	137.16	146.16	105.56
127	164.04*	102.82	181.78	161.42	98.74	162.78*	93.66	157.25	103.81	121.56	105.61	146.26	137.77	146.26	105.61
128	162.89	105.98	183.16	159.79	107.52*	136.04	111.05*	155.83	110.11	129.43	128.41	129.22	137.12	129.22	128.41
129	165.48	101.21	182.48	159.90	107.09*	135.22	110.61*	155.67	109.61	116.15	128.46	113.46	153.28	113.46	128.46
159	146.69	135.64	175.74	160.62	98.09	163.79	93.26	156.04	102.91	121.87	115.51	144.95	147.66	115.51	119.89
160	78.37	41.98	196.00	163.38	95.66	166.53	94.86	162.81	101.70	129.36	114.25	145.10	145.92	115.24	117.86
161	82.95	71.45	197.90	163.22	95.88	166.68	94.88	162.46	100.38	127.93	115.00	144.83	145.67	115.25	119.31
162	153.05	123.06	174.52	124.15	115.09	162.48	102.04	157.36	116.53	124.15	129.99	113.50	158.86	113.50	129.99
163	154.21	121.87	180.02	159.07	98.93	164.26	93.63	157.51	104.37	122.84	130.08	113.62	161.91	113.62	130.08
164	153.89	121.12	180.12	161.91	98.87	164.19	93.57	157.49	104.38	122.19	130.07	114.97	157.33	114.97	130.07

Table 8: Chemical shift of ^{13}C NMR of different hydroxylated flavonoids in ppm in DMSO-*d* [6]

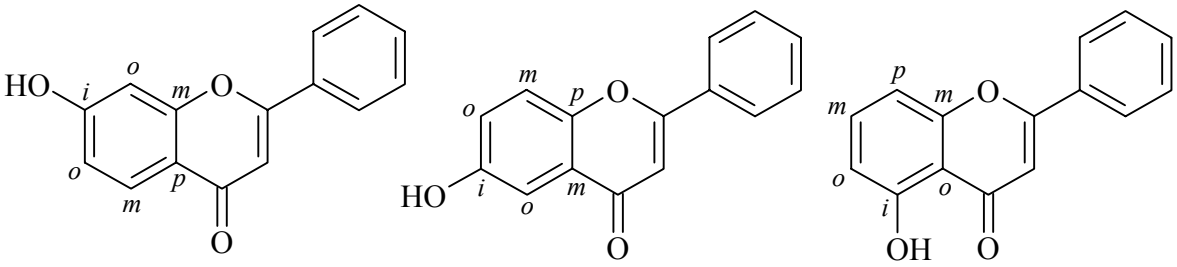
* Values may be switched

13.37±0.15 ppm).

The introduction of a hydroxyl group always has a bigger influence on the carbon atom on which it is linked to (we will call it *ipso*) the other influences are noticed at the *ortho* and *para* positions. For this reason, the chemical shift is more deshielded following the classification of the position:

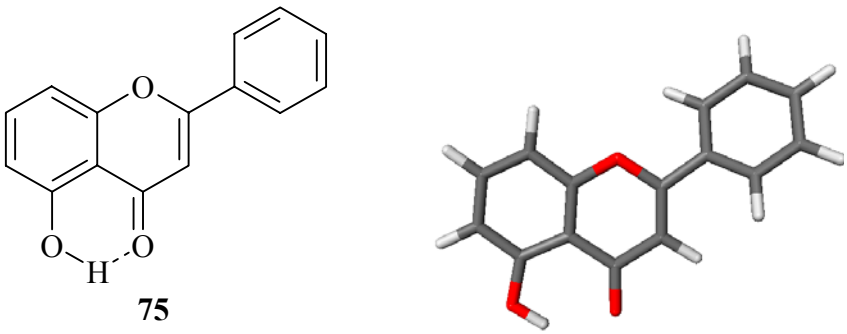
5>6>7

which can be explained by the conformation of the hydroxyflavones (Scheme 2).



Scheme 50: Dependence of the position for the influence on the chemical shifts

The presence of the hydroxy at the 5-position causes 5.89±0.30 ppm downfield shift of the C-4 resonance hence resulting of the appearance of C-4 resonance 182.99±0.11 ppm. It is because of the intramolecular hydrogen bond interactions existing between the keto group (C-4) and C-5 (*peri*)* the hydroxyl group (Scheme 51).



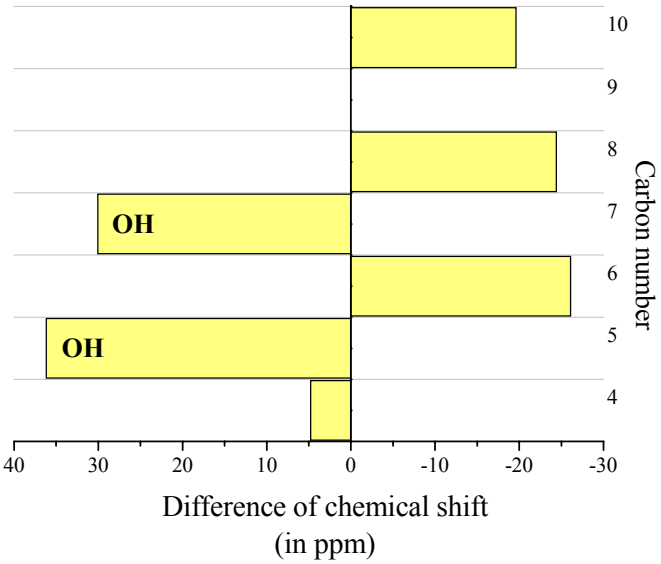
Scheme 51: Intramolecular hydrogen bond interaction on 5-hydroxyflavone (2D and 3D view†)

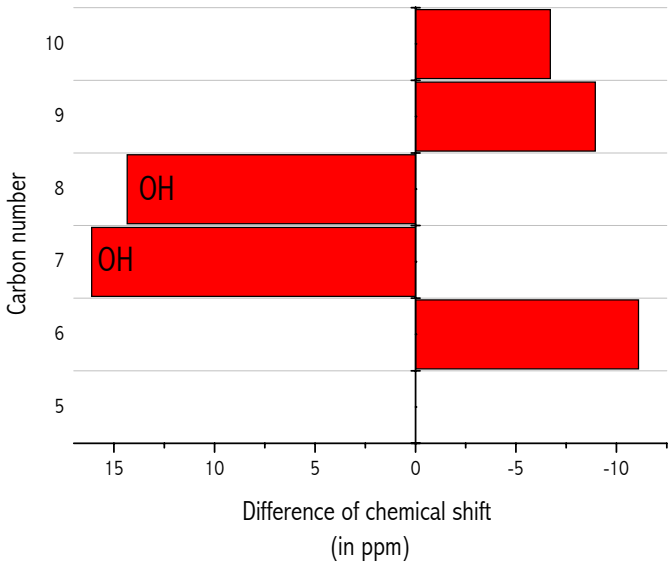
* *peri* is the position of the C-5 considering the C-4 keto group
† Conformation has been calculated by VAMP Software.

The introduction of one hydroxyl group on the A-ring has negligible effects on the chemical shifts of the carbon atoms of the B-ring.

2.1.1.2. Introduction of several hydroxyl groups

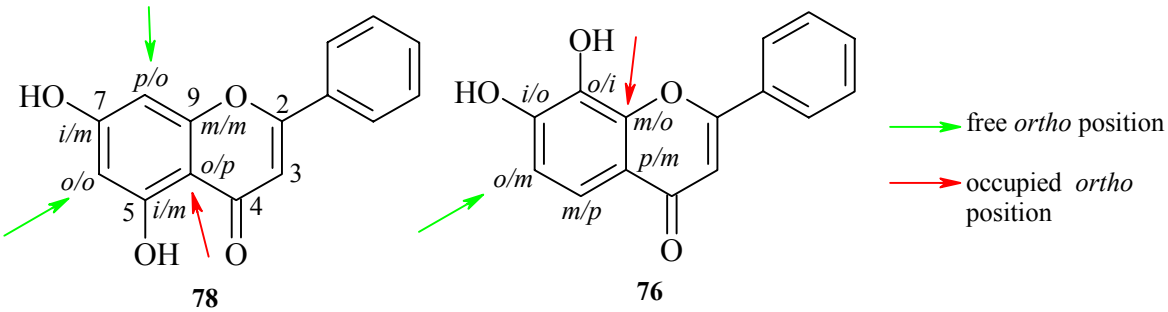
We consider now the introduction of two and three hydroxyl groups on the A-ring. The introduction of hydroxyl groups at 5- and 7-position induces perturbation ▯ of the chemical shifts of following carbon atoms: 4 (+4.79±0.03 ppm), 5 (+36.18±0.29 ppm), 6 (–26.08±0.25 ppm), 7 (+30.07±0.16 ppm), 8 (–24.40 ±0.04 ppm) and 10 (–19.60±0.15 ppm) of the flavones **78**, **87**, **95**, **103**, **111**, **119** and **127** in comparison with the corresponding unsubstituted flavones (**24**, **80**, **88**, **96**, **104**, **112** and **120**). If we change now the position of the introduced hydroxyl groups, like in the 7,8-dihydroxyflavones (**76**, **85**, **93**, **101**, **109**, **117** and **125**) in comparison with the flavones **24**, **80, 88**, **96**, **104**, **112** and **120,** a perturbation ▯ of the chemical shifts can be observed on the following carbon atoms: 5 (–9.73±0.56 ppm), 6 (–11.09±0.33 ppm), 7 (+16.11±0.56 ppm), 8 (+14.35±0.23 ppm), 9 (–8.94 ±0.07 ppm) and 10 (–6.71±0.42 ppm).





We can observe the difference between two hydroxyl groups with two free ortho positions (**78**) and two hydroxyl groups with one free ortho position (**76**) (Scheme 52). The chemical

shift of the first kind of hydroxyl is shifted more downfield than the second kind of hydroxyl groups.



Scheme 52: Influence of the substituent positions on the A-ring

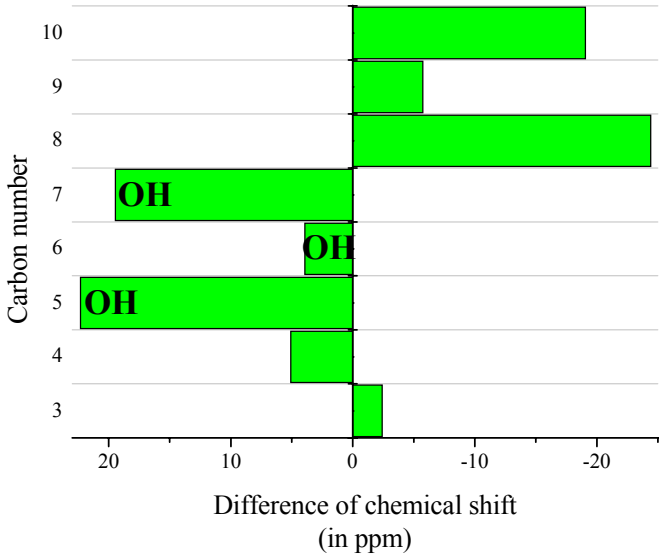
The Scheme 52 shows effectively that in the case of the 5,7-dihydroxyflavone, if we consider one hydroxyl group at the 5- or 7-position, the second hydroxy group (at the 7- or 5-position) does not influence any *ipso* position, because the *ipso* position of one hydroxyl group represents the *meta* position for the second one. The carbon atom at the 6-position is, in this case, at a common *ortho* orientation and is subject to the double influence of both hydroxyl groups hence the unusual upfield. The 8- and 10-positions are a common to the *para* and *ortho* orientation of each hydroxyl groups and are subject to an important upfield shift.

In the case of the 7,8-dihydroxyflavone, we can notice that the shift will be smaller than in the 5,7-dihydroxyflavone. Most of the positions are a combination of a *meta* and *ortho*, or a *meta* and *para* positions. Thus, even if we are in presence of two hydroxyl groups on the A-ring the effects on the chemical shifts of C-5 and C-6 are the same as those induced by only one hydroxyl group on this ring. An exception can be shown for the *ipso* positions, which are combined with a *ortho* orientation and shift less downfield the chemical shift of the carbon atoms 7 and 8. After these remarks, we can set a classification of the influence of the combination of several positions (absolute value):

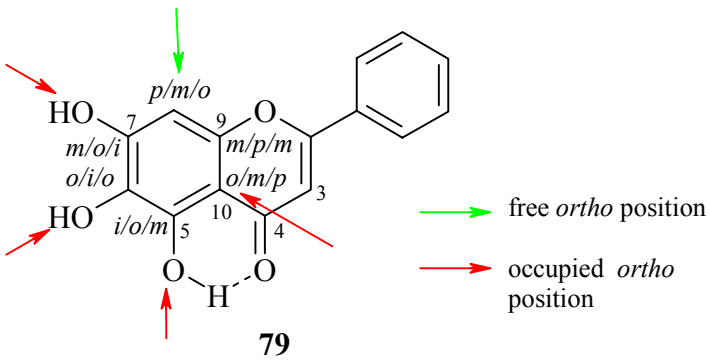
$$m/m < m/p < m/o < i/o < p/o < o/o < i/m$$

The introduction of several hydroxyl groups on the A-ring has negligible effects on the chemical shifts of the carbon atoms of the B-ring.

We considered the 5,6,7-trihydroxyflavone (**79**) in comparison to the flavone (**24**). The introduction of three hydroxyl groups affected most of the chemical shifts ▮ of carbon atoms:



3 (–2.41 ppm), 4 (+5.08 ppm), 5 (+22.30 ppm), 6 (+3.93 ppm), 7 (+19.46 ppm), 8 (–24.41), 9 (–5.74 ppm) and 10 (–19.06 ppm). We can explain the origin of such a variation of the chemical shifts by the combination of the positions *ipso*, *ortho*, *meta* and *para* (Scheme 53). The 5,6,7-trihydroxyflavone (**79**) possesses three withdrawing groups that affect most of the chemical shifts of the carbon atoms of the A- and C-rings. For the carbon atoms of the C-ring, this variation of chemical shifts is due to the hydroxyl group at the 5-position because of its *peri* position, which causes intramolecular hydrogen bond interactions with the keto group C-4 shown as a downfield shift. These intramolecular interactions lead to an impoverishment of the C-3, which manifests itself by an upfield shift.



79

Scheme 53: influence of the substituent positions on the A-ring

The atoms C-5 and C-7 are at a position, which is a combination of *ipso*, *meta* and *ortho* position, hence the important downfield shift. Between them, the carbon 6 is at the combination of two *ortho* and one *ipso* positions leading to a weak downfield shift. The C-8 is

at the only free *ortho* position, in combination with a *para* position that is why it is subject to the bigger upfield shift compared to the C-10, which is at an occupied *ortho* position. At least the weaker influence of the chemical shift is observed for the C-9, which is at the combination of two *meta* and one *para* positions, the same effect as one *para* position.

We can establish a scale of the influences of position combinations on the chemical shift (absolute value):

p/m/o (free)> i/o/m > p/m/o (occupied) > m/p/m > o/i/o

2.1.2. B-ring

2.1.2.1. Introduction of hydroxyl groups

The introduction of one hydroxyl group on the B-ring strongly influences the *ipso* carbon atom and the carbon atoms at the *ortho* and *para* positions (Figure 1), as we already quoted the effect of a hydroxy group on the A-ring. In the case of flavones **104**, **108**, **107**, **106**, **111**, **109** with one hydroxyl group at the 4′-position and their corresponding unsubstituted B-ring flavones **24**, **75**, **74**, **73**, **78** and **76**, the perturbation of the *ortho* carbon atoms (C-3′ and C-5′), *meta* carbon atoms (C-2′ and C-6′), and *para* carbon atom (C-1′), is –13.18±0.04 ppm, +2.04±0.13 ppm and –9.44±1.26 ppm, respectively. The oxygen atom affects the chemical shift of its directly linked carbon atom with a downfield shift by +25.55±4.07 ppm, as we already remarked in the case of the introduction of a hydroxyl group on the A-ring.

We considered then the flavones **112**, **116**, **115**, **114**, **119** and **117**, which have two hydroxyl groups at the 3′- and 4′-position of the B-ring in comparison to the unsubstituted flavones **24**, **75**, **74**, **73**, **78** and **76** (Figure 1). The carbon atom C-3′ and C-4′ are shielded by +20.35±0.32 ppm and by +13.92±0.27 ppm and their corresponding *ortho* neighbours (C-2′ and C-5′) are deshielded by –12.63±0.64 ppm, then the *meta* carbon atoms (C-6′ and C-1′) are deshielded by –7.89±1.34 ppm and –9.18±1.14 ppm, respectively. We recognize in Scheme 54 again a case of the catechol on the B-ring and the variation of the electronic effects of both

hydroxyl groups depending on their orientations (*ipso*, *ortho*, *meta* and *para*). The combinations with the position *meta* are the major ones and affect the carbon atoms as if only one hydroxyl group were on the B-ring excepted for the *ipso* positions, which are less subject to a downfield shift.

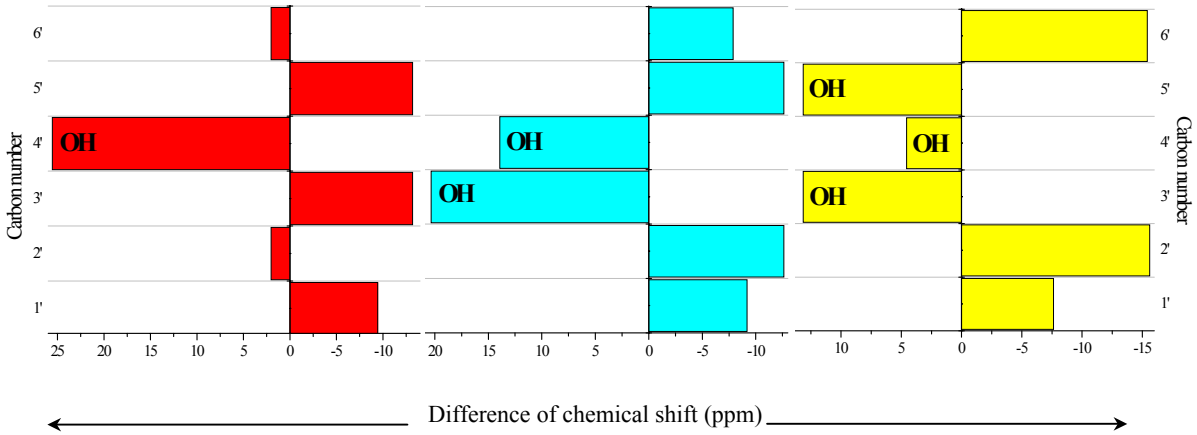
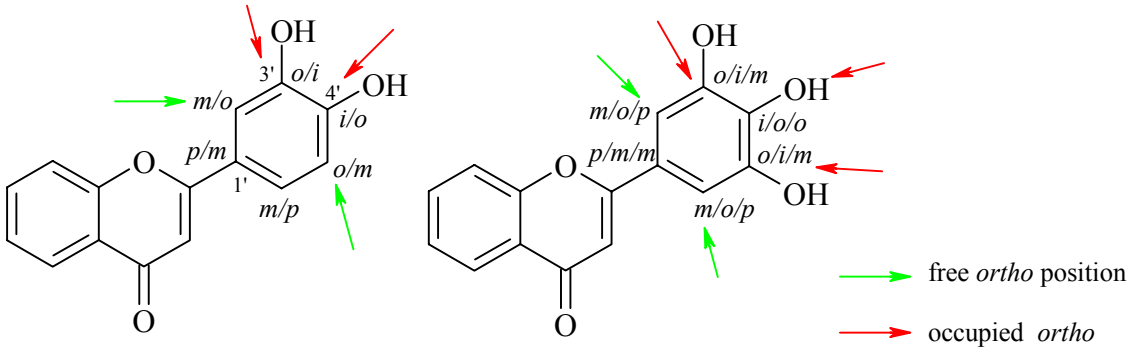


Figure 1: Variation of the chemical shift of carbon atoms of the B-ring after the introduction of one, two or three hydroxyl group(s)

By considering the flavones **120**, **122** and **127** with three hydroxyl groups on the B-ring and the unsubstituted flavones **24**, **73** and **78**, the perturbation due to the introduction of three hydroxyl groups affect the following atoms: 1′ (–7.62±3.37 ppm), 2′ and 6′ (–15.41±7.43 ppm), 3′ and 5′ (+13.13±6.61 ppm), and 4′ (+4.55±2.32 ppm).

In Scheme 54, we can identify the different combinations of the three hydroxyls groups: only the 4′-position is not a combination of a *meta* orientation but one *ipso* with two *ortho,* which does explain the small shielding of the chemical shift even if a hydroxyl group is linked to the carbon atom. The carbon atoms C-3′ and C-4′ are not subject to an important downfield shift because they are at a combination *ortho*, *ipso* and *meta*, like in the case of the carbon atoms of the 3′,4′-dihydroxyflavone. At least the biggest influence on the chemical shift of the carbon atoms happens for the C-2′ and C-6′, which are at a *meta*, *para* and *ortho* combination, hence the upfield shift.



Scheme 54: Influence of the position of the substituents on the B-ring

As we set a classification for two hydroxyl groups, we can here again set a classification (absolute values) of the influence of the position of the hydroxyl groups of the ring, on the chemical shifts of the carbon atoms:

$$i/o/o < p/m/m < o/i/m < m/o/p$$

The introduction of several hydroxyl groups on the B-ring has negligible effects on the chemical shifts of the carbon atoms of the A- and C-rings.

2.1.2.2. Introduction of methoxyl groups / methylation of hydroxyl groups

Most of our synthetic flavones occur as methyl ethers possessing methyl substituent alkylated to one or more hydroxyl group(s) on the B-ring. Methylation of hydroxyl group causes a change in ^{13}C shielding on a few carbons, which in comparison with hydroxy equivalent are referred as methylation induced shift. Methylation also introduces an extra signal for each methoxyl carbon. Methyl carbons appear in two distinct chemical shift ranges, 55-56.5 ppm and 59-63 ppm: the methoxyl group with at least one free *ortho* position usually referred as "normal methoxyl" appears in the former chemical shift range whereas, sterically hindered methoxyl group with the substituent in both ortho positions resonates in the later chemical shift range. The chemical shift difference between the above-mentioned two type of methoxy groups is due to the fact that the normal methoxyl group possesses coplanar* aryl-*O*-methyl bond in which the π orbitals of the aromatic ring tend to overlap with the lone pair

* Is coplanar in relation to the phenyl ring

electron orbitals of the methoxy oxygen, leading to a delocalisation of the non-bonding oxygen electrons and strengthening aryl-*O* bond (Figure 2). This also results in increased electron density at the aryl ring carbons *ortho* and *para* to the methoxy group. In the "out of plane" conformation, which is expected for the di-*ortho* substituted methoxyl, the conjugation of the non-bonding oxygen electrons with the bond of the ring is interrupted, hence resulting to the decreased electron density on methoxyl, ortho and para carbons. The distortion angle of 86° (θ) has been reported[122,123] for "out of plane" methoxyl group. These interferences are supported by the T1 values for the methoxy resonances[123,124] and theoretical calculations.[125]

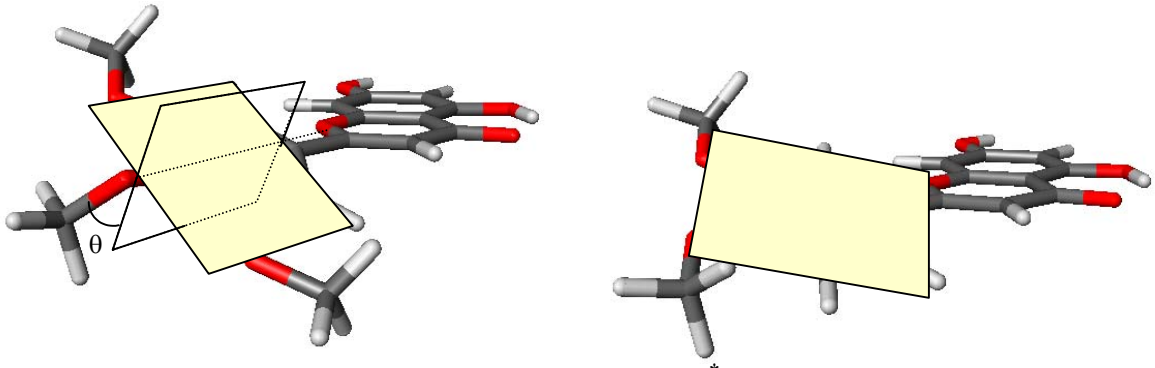


Figure 2: non-coplanar and coplanar aryl-*O*-bond* for the 3′,4′,5′-trimethoxy-5,7-dihydroxyflavone and the 3′,4′-dimethoxy-5,7-dihydroxyflavone

Conversion of a phenolic hydroxyl to a methoxyl group is associated with characteristic change in chemical shifts of the aromatic carbon nuclei of the ring involved. Generally the chemical shift of the *ipso* carbon is deshielded by 3.05±1.42 ppm and *ortho* carbons get upfield shifted by 1.36±0.04 ppm. *Para* carbon atom gets usually deshielded by about 1.30±0.42 ppm whereas *meta* carbons remain more or less unaffected. Methylation induced shift values exhibit dependence to the site of the hydroxyl group as well as to the presence of ortho substituents.

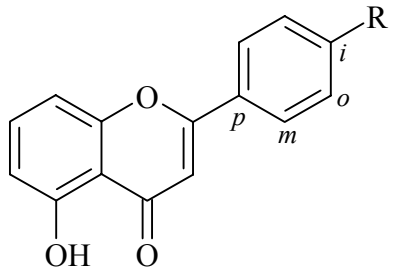
In case of *ortho* oxysubstituted phenols, *O*-methylation causes an upfield shift by an average of 0.86 ppm for the substituted *ortho* carbon. In *ortho* disubstituted phenols methylation lead to the downfield shifts of varying magnitudes +2.89±0.11, +4.21±0.33 and

* Conformations have been calculated by VAMP software.

+1.13±0.65 for the *ipso*, *ortho* and *para* resonances, respectively.

2.1.2.3. Nature of the substituents

If we change the nature of the substituents at the 4′-position on the B-ring (flavones **75**, **84**, **108**, **128** and **130** in Table 9), we can notice that polar groups directly attached to the benzene ring cause upfield and downfield shifts more and less in the same way that they would do with a proton.



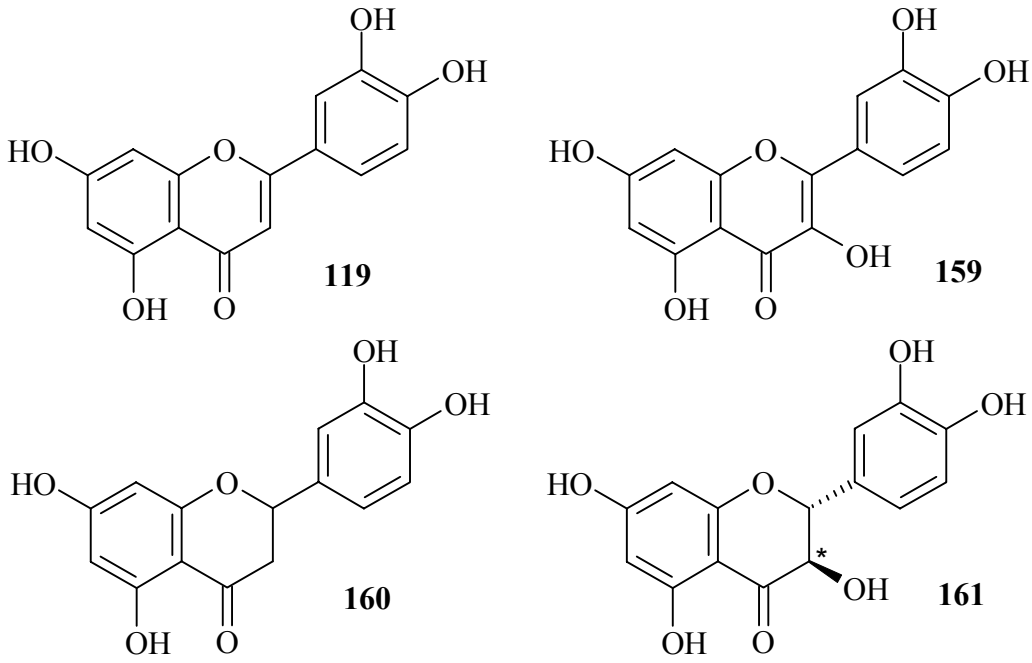
$\Delta\delta_c$	*p*	*m*	*o*	*i*
R=OMe	8,40	-2,39	14,40	-30,89
R=OH	10,17	-2,45	13,06	-28,11
R=Cl	1,59	-2,19	-0,25	-5,45
R=NH_2	14,87	-2,24	15,51	-21,61

Table 9: $\Delta\delta$ of the carbon atoms (in ppm)

The effects of a π-donor and a π-acceptor are seen in Table 9, where the signals of the *para* and *ortho* carbons are shifted upfield by the methoxy, hydroxy or amino groups relative to the signal of flavone **75**. The chloride atom is a weak σ-acceptor and weak π-donor and shows a complete different influence on the *ipso*, *ortho* and *para* positions.

2.2. Flavonol, Flavanone, Flavanonol

In the paragraph about flavones, we studied the structure change on the A- and B-rings of the skeleton. Now we present some changes on the C-ring: the effects of the introduction of a hydroxyl group at the 3-position, and the suppression of the double bond between C-2 and C-3 in comparison to Luteolin (**119**). For this reason, we selected Quercetin (**159**), Eriodictyol (**160**), and Taxifolin (**161**), representative examples of Flavonol, Flavanones and Flavanonol families respectively.



Scheme 55: Chemical Structure of Luteolin (**119**), Quercetin (**159**), Eriodictyol (**160**) and Taxifolin (**161**)

Flavanones, like Eriodictyol, possess a 2-phenylchromanone as the parent skeleton. Since C-2 of the Flavanonol, like Taxifolin, is a centre of asymmetry, two isomeric forms of each structure are possible but most of the naturally occurring flavanones acquire phenyl substituent at C-2 position in pseudoequatorial orientation as indicated on the structure (**161**). Thus the heterocyclic C ring is characterized by the three resonances of: an oxymethine (C-2), an aliphatic methylene (C-3) and a carbonyl (C-4) which present three different oxidation degrees.

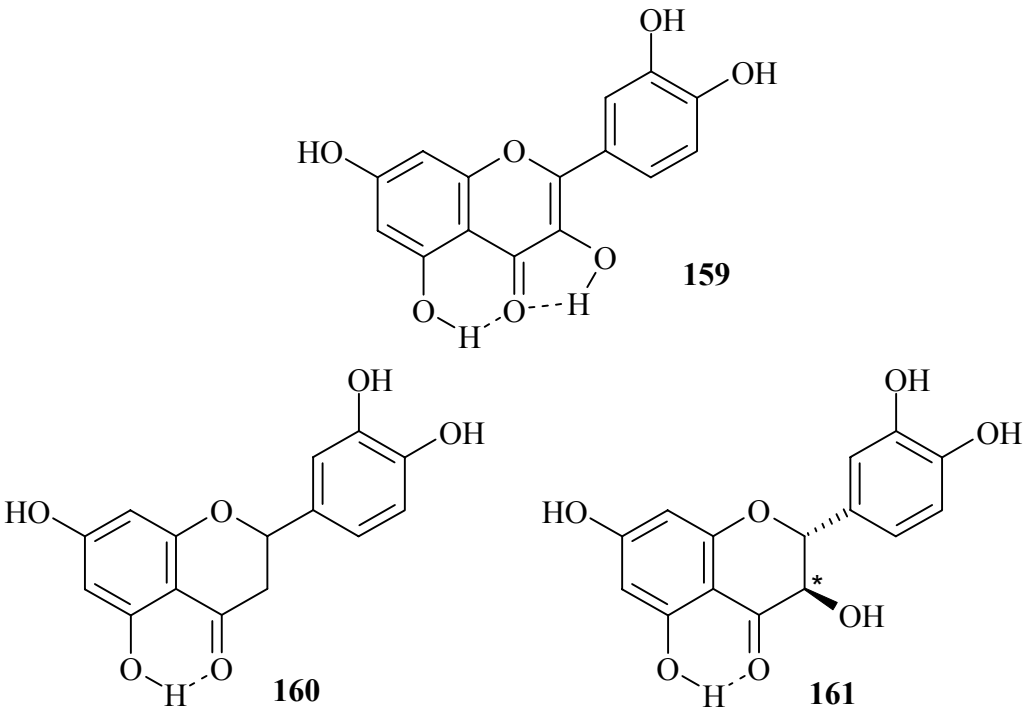
2.2.1. Introduction of 3-OH

The chemical shift of C-2 and C-3 are dramatically affected by the introduction of the 3-hydroxyl substituent into the flavonoid skeleton. A hydroxyl substitution at C-3 of flavanone leads to about 4.6 and 29.5 ppm downfield shifts of the C-2 and C-3 resonances respectively while it leads to about 17 ppm upfield and 42 ppm downfield shifts of the C-2 and C-3 resonances of flavone. The C-4 resonance remains almost influenced and moves upfield by 6 ppm in going from flavanone to flavanonol or from flavone to flavonol.

The C-3 and C-2 shifts downfield by 29 and by 17 ppm and C-4 moves upfield by 5 ppm in going from flavone to flavonol. We can also notice that the C-6 and C-8 resonance are shifted downfield by 5 and 6 ppm.

2.2.2. Double bond between C-2 and C-3

In relation to Eriodictyol, the C-2 and C-3 of Luteolin are dramatically deshielded by 85 and 52 ppm, because of their sp^2 nature. The presence of the 2,3-olefinic bond also leads to an upfield shift 14.4 ppm of the carbonyl resonance (C-4). In the comparison Quercetin of into Taxifolin, both C-2 and C-3 are quaternary oxy-olefinic type and change to methine type with a upfield shift by 64 ppm on both carbon resonances, that is explained by the equal repartition of the electron density on the π-bond/orbital which is not the case in the flavone/flavanone with their two different kinds of carbon nature. The C-4 resonance moves downfield by 22 ppm.

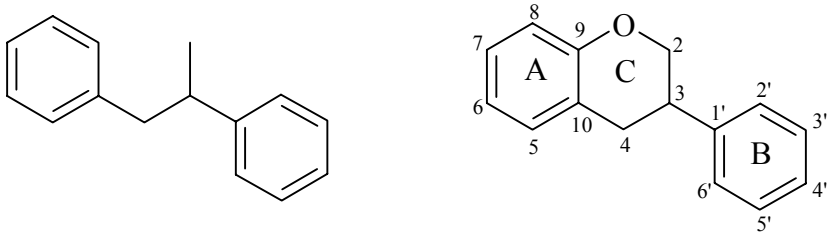


Scheme 56: Intramolecular hydrogen bond interactions in flavonoids

The 5-hydroxy substitution causes in all flavonoid some intramolecular hydrogen bond interactions with the keto group (C-4)[126]. Furthermore, for Quercetin the 3-hydroxyl group takes also part to a hydrogen bonding and enhances the interactions (Scheme 56).

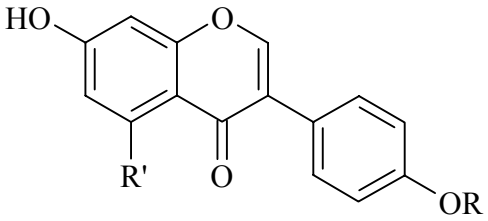
2.3. Isoflavones

Isoflavonoids are biogenetically related to flavonoids but constitute distinctly a separate class, in that they contain a rearranged C_{15} skeleton and can be considered as derived from the 1,2-diphenyl propane (Scheme 57). The isoflavones are the 2,3-unsaturated 4-oxo derivatives of the isoflavan structure. Thus, these possess a 3-phenylchromone skeleton.



Scheme 57: Chemical structure of 1,2-diphenyl propane and isoflavan skeleton

The heterocyclic ring C in isoflavones is constituted of an oxyolefinic methine (C-2), olefinic quaternary carbon (C-3) and a carbonyl carbon (C-4). On the examples of Formononetin, Biochanin A, and Genistein (Scheme 58), these carbons (C-2, C-3 and C-4) have a chemical shift of 153.05-154.21, 121.12-123.06, and 174.52-180.02 ppm respectively.

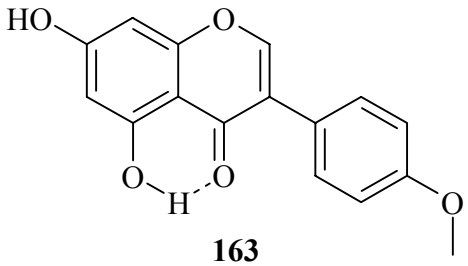


162 Formononetin R' = H, R = Me
163 Biochanin A R' = OH, R = Me
164 Genistein R' = OH, R' = H

Scheme 58: Chemical structure of the studied isoflavones

The chemical shift of the C-2 remains relatively unaffected by the substituent in the aromatic rings. However, chemical shifts of C-3 and C-4 exhibit a dependence upon the substitution in aromatic ring B as well as ring A, particularly with an oxy substituent. The presence of hydroxyl group at the 5-position influences the chemical shift of the C-ring carbon resonance through intramolecular hydrogen bond interactions (Scheme 59). Thus C-4 appears between 180.00-181.00 ppm in 5-hydroxylated isoflavones, while the carbonyl group resonates at 174.52 ppm in 5-unsubstituted isoflavones. Hence, C-4 absorbs at a 5-7 ppm

deshielded position in 5-hydroxy isoflavones. Another consequence of the presence of one hydroxyl group at the 5-position is the appearance of C-2 resonance at a 3 ppm deshielded position. It is because of the withdrawal of electrons towards the chelated carbonyl group due to the intramolecular hydrogen bond interaction.



163

Scheme 59: Intramolecular hydrogen bond interaction in isoflavone

3. Conclusion

The assignment of carbon nuclear magnetic resonances of flavonoids for their characterisation can prove difficult because all subcategories of the flavonoids differ in the nature of carbon atoms constituting the parent skeleton, therefore a knowledge of the types of the carbon presents in the flavonoid under investigation may provide information about the skeletal type and extent of aromatic substitution.

The introduction of several group on the skeleton of flavonoids showed a variation of influence on the chemical shift of the carbon atoms, due to the combination of the orientation (*ipso*, *ortho*, *meta* and *para*) pattern of the introduced groups, which leads to a classification of the influence following the number of introduced groups. Hence an easier assignment of ^{13}C NMR spectra as the both parts of the skeleton (A- and C-rings on one side and the B ring on the other side) have none or a very weak influence on the chemical shifts of each part.

We saw that polysubstitutions at characteristic positions on the skeleton can influence the planar aspect of the skeleton or of its aryl-*O*-bond that leads to a change of the electron density of the compound, hence the conformation, the physical and/or chemical properties.

Chapter 5: Structure-UV Activity Relationship

1. Introduction

Vogt *et al*[127] observed that flavonoids might be concentrated either at the leaf surface or intracellularly in the epidermal cells. This observation has led to the oft-repeated suggestion that they contribute to the protection of plant tissues from damaging UV radiation. Thus, the surface components could protect from UV-B (280-315 nm) radiation, while intracellularly constituents filter our UV-A (315-350 nm) radiation. Evidence in support of this hypothesis has been obtained by irradiation experiments on the Mediterranean scrub *Cistus laurifolius*, which has both types of leaf flavonoids. Indeed, UV-A radiation increases the concentration of glycoside in the cell vacuole two-fold over the control, while the amounts of epicuticular aglycones were unaffected. Markham *et al*[128] suggested that the concentration of flavone glycosides in the Antarctic moss *Bryum angenteum* varies annually, increasing or decreasing according to the intensity of the UV radiation at any one-year.

Natural flavones (bearing either hydroxyl or methoxy groups or linked to a sugar residue) can play a role as UV-filter, which has been observed and established. Due to their importance, the absorption spectra of flavonoids have been studied in great detail,[129,130] since absorptiometry is one of the most suitable methods for the structure elucidation and identification of flavonoids.

2. UV Absorption spectra

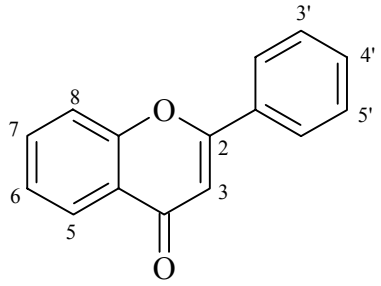
The UV spectrum of flavonoids is usually determined in ethanol or methanol and typically consists of two absorption maxima in the ranges of 240-285 nm (referred as Band II, primarily due to the A-ring absorption), and 300-550 nm (referred as Band I, due to the B-ring absorption). The precise position and relative intensities of these maxima give valuable information regarding the nature of the flavonoid and its oxygenation pattern. A guide to the principal maxima for each studied flavonoids is given in Table 10.

Name	Minima (nm)	Maxima (nm)	Name	Minima (nm)	Maxima (nm)
Flavone	251	293.5	4′-hydroxyflavone	254 (sh)	312(sh), 328.5
8-hydroxyflavone	*n.d.*[a]	*n.d.*[a]	4′,8-dihydroxyflavone	*n.d.*[a]	*n.d.*[a]
7-hydroxyflavone	250	308	4′,7-dihydroxyflavone	231, 254	313 (sh), 329
6-hydroxyflavone	272	302.5	4′,6-dihydroxyflavone	228, 277	328
5-hydroxyflavone	270.5	298, 336.5	4′,5-dihydroxyflavone	271	321.5
5,7-dihydroxyflavone	269.5	315.5	4′,5,7-trihydroxyflavone	269	339
6,7-dihydroxyflavone	267	311	4′,6,7-trihydroxyflavone	*n.d.*[a]	*n.d.*[a]
7,8-dihydroxyflavone	268.5	318.5	4′,7,8-trihydroxyflavone	270.5	325
5,6,7-trihydroxyflavone	276.5	325.5	3′,4′-dihydroxyflavone	244.5	309, 353
4′-methoxyflavone	252	316.5	3′,4′,8-trihydroxyflavone	*n.d.*[a]	*n.d.*[a]
4′-methoxy-8-hydroxyflavone	*n.d.*[a]	*n.d.*[a]	3′,4′,7-trihydroxyflavone	237	311 (sh), 342
4′-methoxy-7-hydroxyflavone	256	323	3′,4′,6-trihydroxyflavone	251, 277	339
4′-methoxy-6-hydroxyflavone	278	322	3′,4′,5-trihydroxyflavone	252	355
4′-methoxy-5-hydroxyflavone	271	321.5	3′,4′,5,7-tetrahydroxyflavone	256, 268 (sh)	313 (sh), 354
4′-methoxy-5,7-dihydroxyflavone	270	327	3′,4′,6,7-tetrahydroxyflavone	*n.d.*[a]	*n.d.*[a]
4′-methoxy-6,7-dihydroxyflavone	271	321.5	3′,4′,7,8-tetrahydroxyflavone	255, 277	343
4′-methoxy-7,8-dihydroxyflavone	268.5	309	3′,4′,5′-trihydroxyflavone	244.5	309, 346
3′,4′-dimethoxy-flavone	242.5	333.5	3′,4′,5′,5-tetrahydroxyflavone	*n.d.*[e]	*n.d.*[e]
3′,4′-dimethoxy-8-hydroxyflavone	*n.d.*[a]	*n.d.*[a]	3′,4′,5′,5,7-pentahydroxyflavone	*n.d.*[c]	*n.d.*[c]
3′,4′-dimethoxy-7-hydroxyflavone	236.5	332	3′,4′,5′,6,7-pentahydroxyflavone	*n.d.*[e]	*n.d.*[e]
3′,4′-dimethoxy-6-hydroxyflavone	249.5, 277	331	3′,4′,5′,7,8-pentahydroxyflavone	*n.d.*[c]	*n.d.*[c]
3′,4′-dimethoxy-5-hydroxyflavone	248, 269	342			
3′,4′-dimethoxy-5,7-dihydroxyflavone	270	341	Pratol *O*-Glucosyl	-	322
3′,4′-dimethoxy-6,7-dihydroxyflavone	259 [b]	309 [b]	Luteolin *O*-Glucosyl	-	353
3′,4′-dimethoxy-7,8-dihydroxyflavone	264	331	4′-chloro-5-hydroxyflavone	275	337.5
3′,4′,5′-trimethoxyflavone	240	357	4′-nitro-5-hydroxyflavone	*n.d.*[e]	*n.d.*[e]
3′,4′,5′-trimethoxy-8-hydroxyflavone	*n.d.*[a]	*n.d.*[a]	4′-amino-5-hydroxyflavone	267.5	381
3′,4′,5′-trimethoxy-7-hydroxyflavone	*n.d.*[d]	*n.d.*[d]	Quercetin	256	302, 374
3′,4′,5′-trimethoxy-6-hydroxyflavone	*n.d.*[d]	*n.d.*[d]	Taxifolin	291	335
3′,4′,5′-trimethoxy-5-hydroxyflavone	273.5	329	Eriodictyol	290	335
3′,4′,5′-trimethoxy-5,7-dihydroxyflavone	272	328.5	Formononetin	250	301
3′,4′,5′-trimethoxy-6,7-dihydroxyflavone	-	324	Biochanin A	263	330
3′,4′,5′-trimethoxy-7,8-dihydroxyflavone	270.5	309.5	Genistein	263	331

n.d.: value not determined; [a]: not synthesised; [b]: not validated; [c]: not soluble in isopropanol; [d]: impurities; [e]: not enough amount

Table 10: Absorption maxima and minima for various flavones and flavonoids

Thus, we only gave here the results obtained from the point of view " UV spectra". Study of the curves of absorption in the ultraviolet range of various flavones and of their methoxylated derivatives was done to observe the effect of weak variations of structure. When the flavone was considered (Scheme 60), it can be noticed that the introduction of hydroxyl, methoxy groups, produced on its absorption spectrum different effects that we report in this chapter. The discovery of well determined variations due to given replacements could indeed lead to express laws which, even in a restricted enough domain, would be susceptible to simplify the identification of bioflavonoids isolated from plants.



Scheme 60: Structure of the flavone chromophore

2.1. Flavones

2.1.1. Band (I)

2.1.1.1.Bathochromic effect

Also called red shift, it is a shift of absorption maximum towards longer wavelength. It may be produced by a change of medium, or by the presence of an auxochrome*. In our study, it can be attributed:

- ❖ To the position of hydroxyl groups

We compared some flavones with only one hydroxyl group at different position on the A-ring (Figure 3). When the flavone is considered, we can notice that the introduction of one hydroxyl group at any position already involves a bathochromic effect. Thus, the intensity of the bathochromic effect depends on the position of the hydroxyl group on the A-ring: We observed a red shift of +14 nm, +9 nm and +43 nm for the 7-, 6- and 5-position respectively.

* A substituent on a chromophore that leads to a red shift.

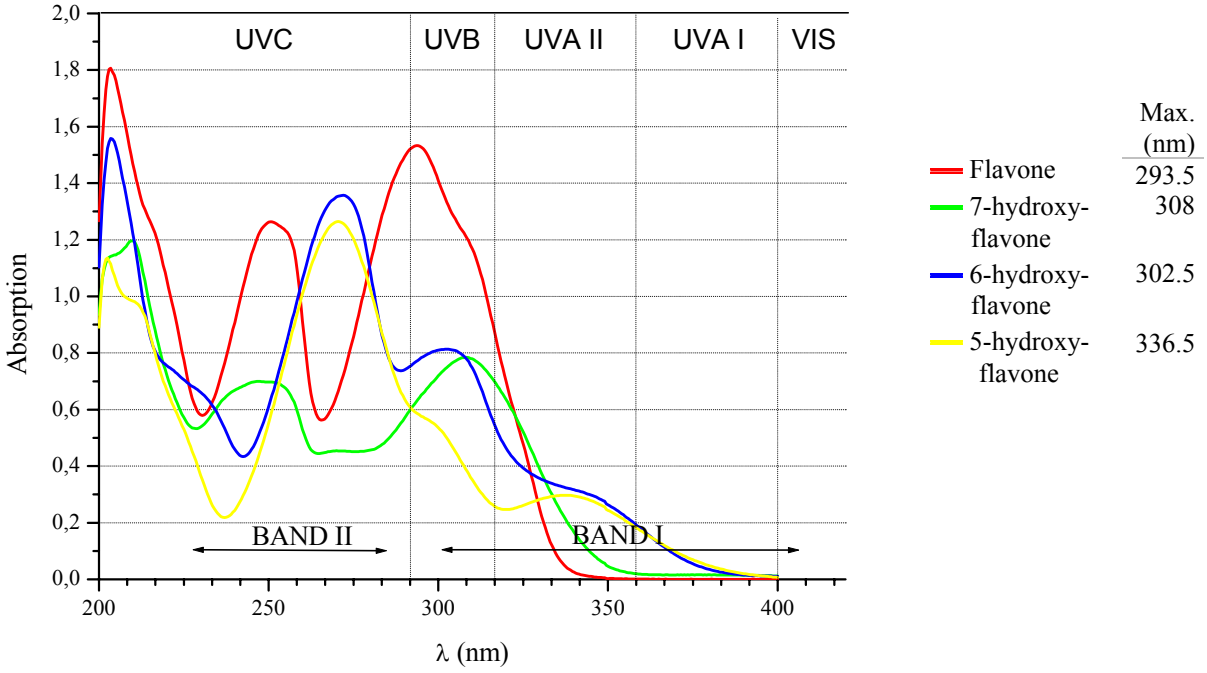


Figure 3: Substitution dependence (position) of the absorption spectra of flavone and monohydroxyflavones in isopropanol. Conc. 10 mM

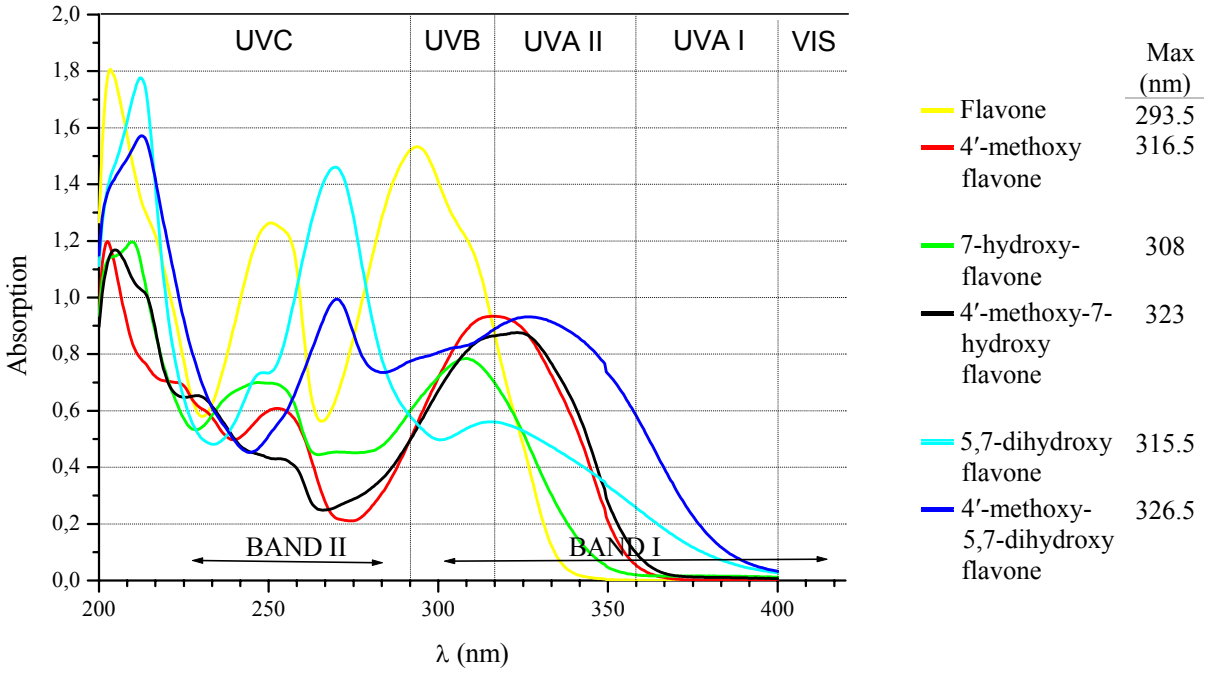


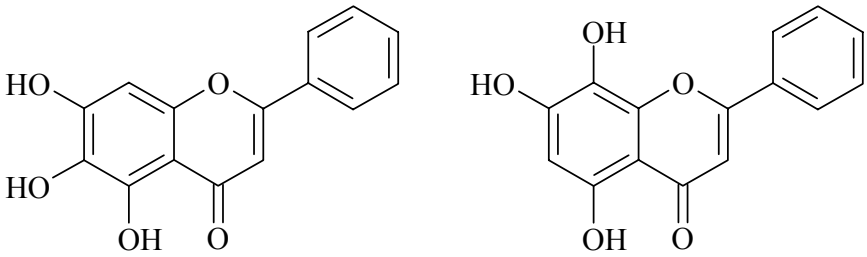
Figure 4: Substitution dependence (on ring position) of the absorption spectra of monohydroxyflavones and methoxyflavones in isopropanol. Conc. 10 mM

We can establish a scale for the influence of the position of the substitution on the bathochromic effect: 6<7<5

The most intense bathochromic effect is observed for the 5-hydroxyflavone (**75**). If we compare now the influence of the position of the hydroxyl group on any rings of the flavone, we can notice that there is an important bathochromic effect of a hydroxyl group even if it is methoxylated at the 4′-position (Figure 4). The observed red shift is respectively +23 nm, +15 nm and +11 nm for the 4′-methoxyflavone, 4′-methoxy-7-hydroxyflavone, 4′-methoxy-5,7-dihydroxyflavone compared to the respective unsubstituted B-ring flavones. Nearby the bathochromic effect, an important hypochromic* effect can be noticed.

❖ To the number of hydroxyl groups:

On one hand, we increase the number of hydroxyl group on the A-ring: it is obvious that the increase of the number of hydroxyl group causes a bigger bathochromic effect accompanied with a small hyperchromic† effect (Figure 5). A first hydroxyl group at the 7-position shows a bathochromic effect of +14 nm compared to the simple flavone, then a second hydroxyl group at the 5-position enhances the effect of the first hydroxyl group of +7.5 nm. If we add a third group the effect is then even more intense, but we noticed that the position where we introduced the third group could have a different influence on the dependence of the absorption spectra. Therefore we compare the 5,6,7-Trihydroxyflavone, also called Bacalein (**79**), to the 5,7,8-trihydroxyflavone[131] (**165**) (Scheme 61).



79 **165**

Scheme 61: Bacalein (**79**) and 5,7,8-Trihydroxyflavone (**165**)

* Hypochromic effect: an effect leading to decreased absorption intensity.
† Hyperchromic effect: an effect leading to increased absorption intensity.

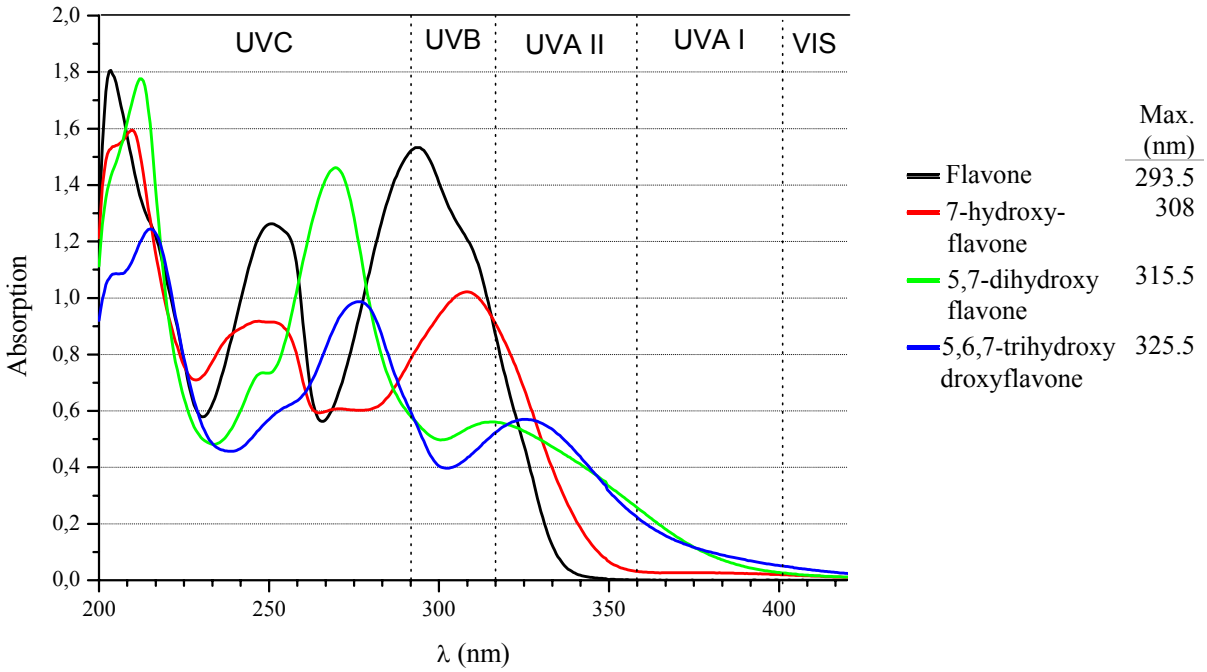


Figure 5: Substitution influence on the absorption spectra of flavone and monohydroxyflavones in isopropanol. Conc. 10 mM

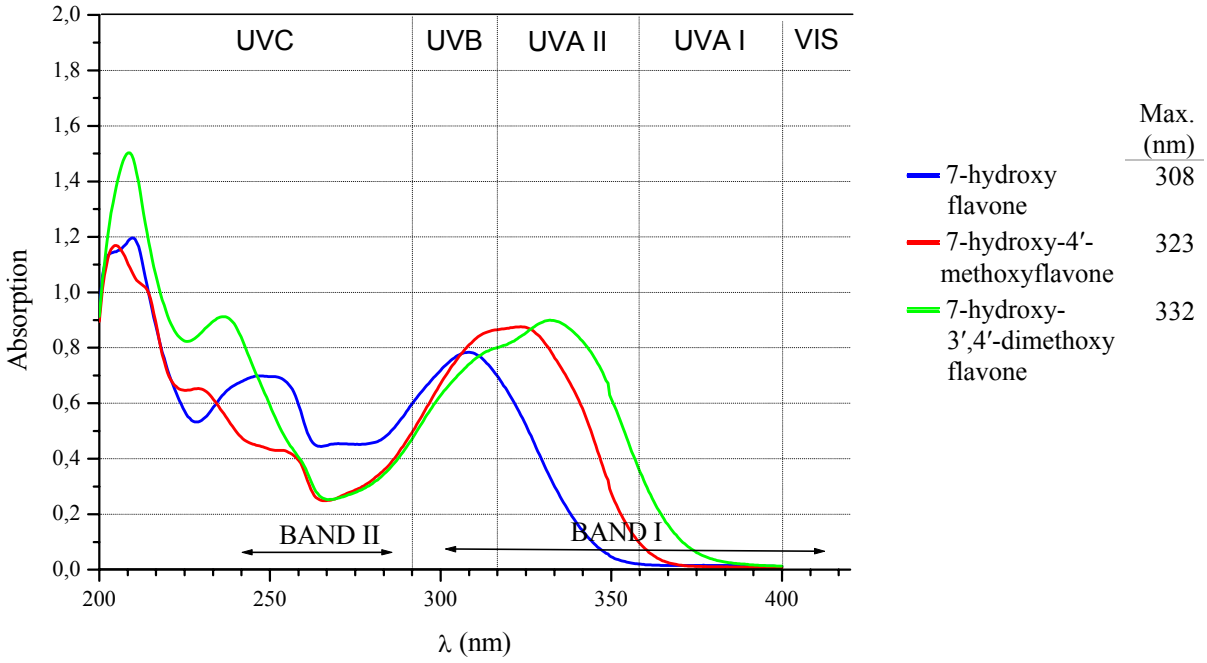


Figure 6: Substitution influence on the absorption spectra of the methoxy substituted B-ring of the 7-hydroxyflavones in isopropanol. Conc. 10 mM

The introduction of the third hydroxyl group at the 6-position enhances the bathochromic effect of +10 nm, which shows an almost linear evolution of the bathochromic effect. The introduction of the third hydroxyl group at the 8-position shifts the absorption maximum almost +50 nm towards the longer wavelength (Figure 7). This effect is considerably intense and shows that the combination of the 5- and 8-positions is more interesting than any other combination.

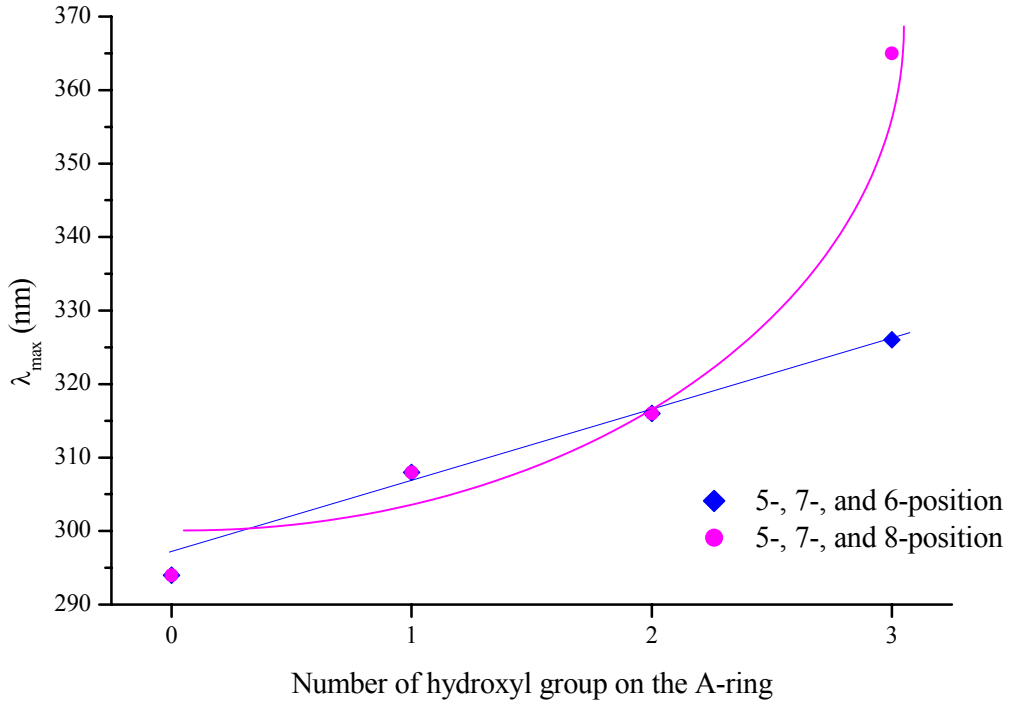


Figure 7: Evolution of the bathochromic effect compared to different substituted flavones

On the other hand, we compare the influence of the substitutions on the B-ring, as most of our synthesised flavonoids possess one to three methoxy groups on the B-ring. In the case of the 4′-methoxy-7-hydroxyflavone we notice a bathochromic effect of +9 nm for the addition of a second methoxy group on the 3′-position and a surprisingly hypsochromic effect of a weak intensity for the introduction of two methoxy group at the 3′- and 5′-position (Figure 6). For the 4′-methoxy-5-hydroxyflavone (**84**) and the 4′-methoxy-5,7-dihydroxyflavone (**87**) (Figure 8 and Figure 9), a bathochromic effect is observed for each introduction of a new methoxy group: A shift of +19.5 nm and +14.5 nm can be measured for the introduction of a

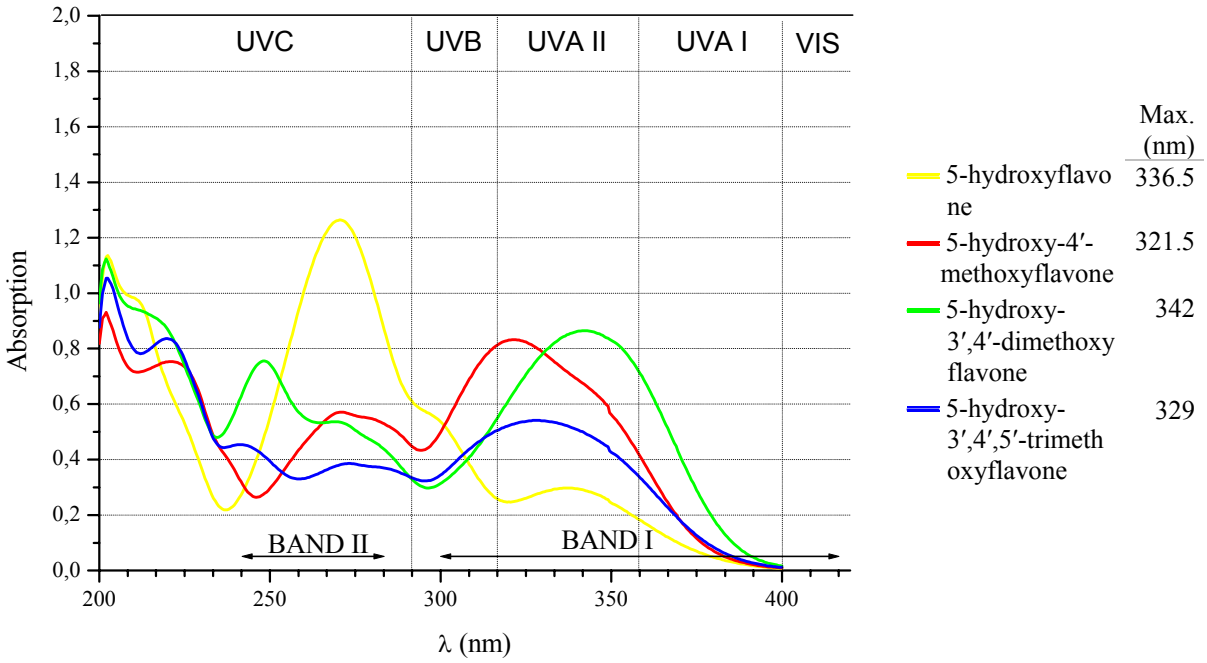


Figure 8: Substitution influence on the absorption spectra of the methoxy substituted B-ring of the 5-hydroxyflavones in isopropanol. Conc. 10 mM

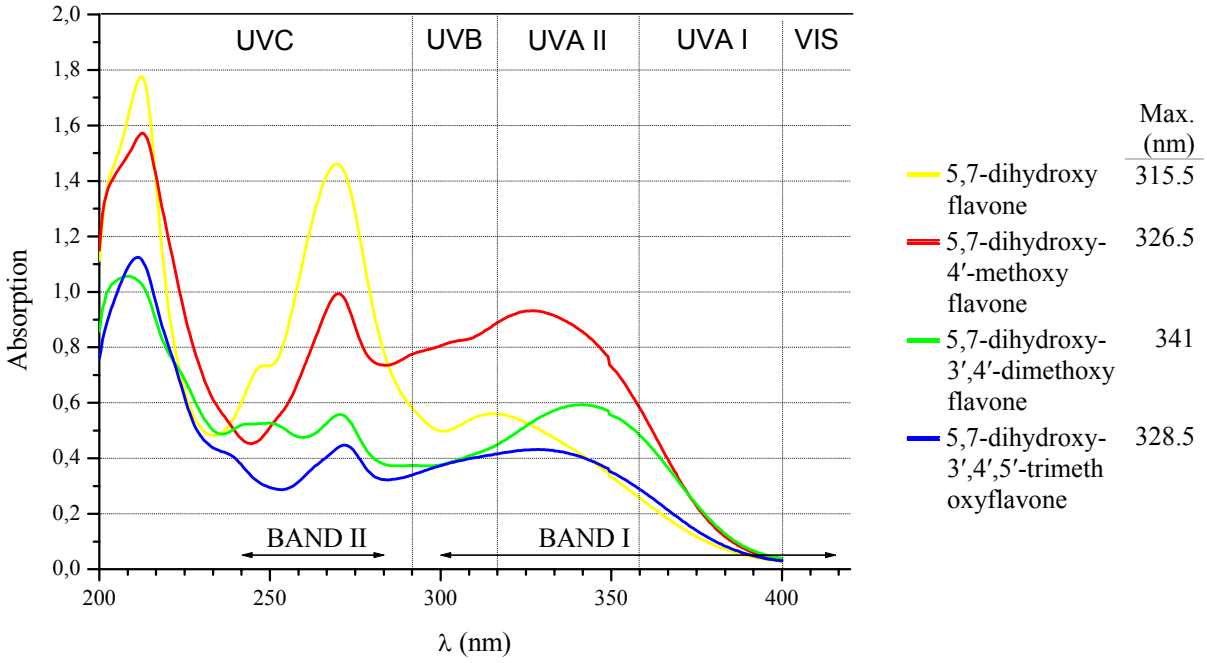


Figure 9: Substitution influence on the absorption spectra of the methoxy substituted B-ring of the 5,7-dihydroxyflavones in isopropanol. Conc. 10 mM

second methoxy group at the 3′-position, and a shift of +7.5 nm and +2 nm for the introduction of two methoxy groups at the 3′- and 5′-positions.

If the number of methoxy groups increases on the B-ring, the bathochromic effect increases for two methoxy groups at the 3′- and 4′-position, and takes the same value if there are three methoxy groups at the 3′-, 4′- and 5′-position as one methoxy group at the 4′-position.

2.1.1.2. Hypsochromic effect

Also called the blue shift, it is a shift towards shorter wavelength. We showed in Figure 10, that the methylation of polyhydroxylated flavones involves a hypsochromic effect: we can notice that the effect is very small (–2 nm) for the methylation of one hydroxyl group, whereas the effect is more intense (–12 nm) for the methylation of two hydroxyl groups.

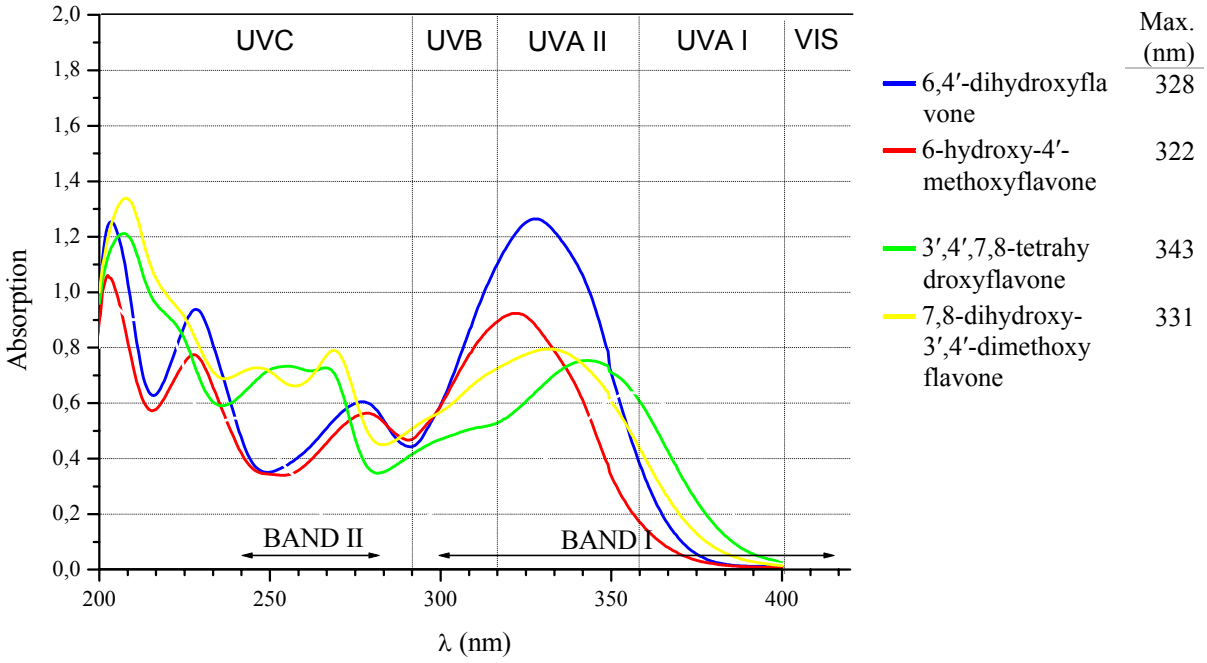


Figure 10: Hypsochromic effect on the absorption spectra of the flavone derivatives in isopropanol. Conc. 10 mM

2.1.2. Band (II)

The effects observed in the Band (II) are less intense than those one in the Band (I). We can distinguish here again both effects.

2.1.2.1. Bathochromic effect

It can be attributed, like in the Band (I), to the position of the hydroxyl group and the number of hydroxyl groups on the flavone.

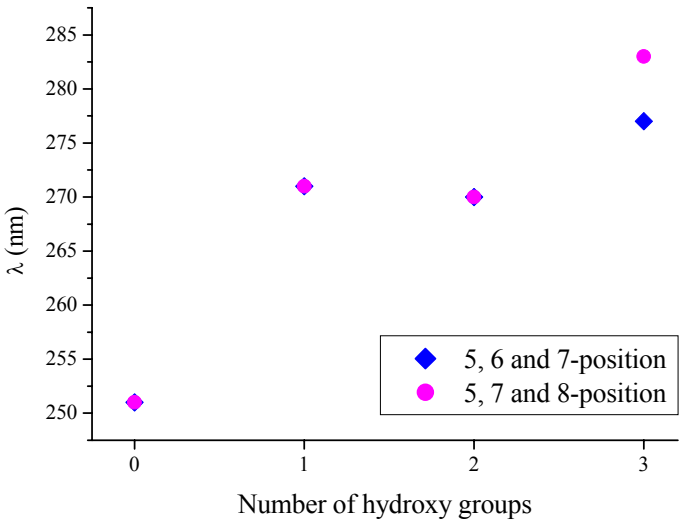
❖ Position of the hydroxyl group

Among all examples already quoted, it is conspicuous that neither a hydroxyl group at the 7-position nor a methoxy group at the 4′-position possess any influence on this Band (II). Only one hydroxyl group at the 5-position can change the value of the maximum of absorption as shown below.

	Max. at
Flavone	251 nm
5-hydroxyflavone	271 nm
7-hydroxyflavone	250 nm
4′-methoxy-5-hydroxyflavone	271 nm
4′-methoxy-7-hydroxyflavone	253 nm

❖ Number of hydroxyl groups

The Figure 11 shows that the bathochromic effect is linked to the number of hydroxyl groups.



	Max.
Flavone	251.0 nm
5-hydroxyflavone	271.0 nm
5,7-dihydroxyflavone	269.5 nm
5,6,7-trihydroxyflavone	276.5 nm
5,7,8-trihydroxyflavone[131]	282.5 nm

Figure 11: Substitution dependence (number) of the absorption spectra of flavones

It confirms that the 7-position has no influence on the shift of the absorption maxima, and that the 5-position is the only one to change the value of the maxima, while the 8-position better enhances more the shift towards the longer wavelength than the 6-position.

2.1.2.2. Hypsochromic effect

While Band (II) is much more difficult to be shifted towards the longer wavelength than Band (I), it possesses an unexpected property:

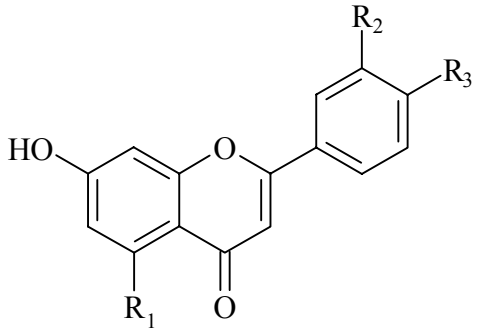
- ❖ When methoxy groups substitute the B-ring at the 3′- and 4′-positions and the flavone has a hydroxyl group at the 7-position, band (II) is shifted towards the UV-C range (λ is 237 nm).
- ❖ When methoxy groups on the B-ring substitute thrice the flavone, the band (II) can either disappear or be affected by a bathochromic effect.
- ❖ Band (III): we know that by taking special precautions, the third band at 200 nm can be observed in the spectrum of flavones. We think that we did observed this third band in the cases of polyhydroxylated flavones, where this band should have come under an intense bathochromic effect.

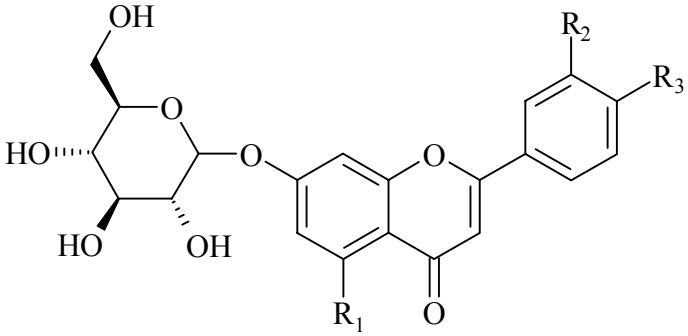
3′,4′-dimethoxy-5,7-dihydroxyflavone	252 nm
3′,5′-dimethoxy-4′,5,7-trihydroxyflavone	243 nm
3′,4′,6-trihydroxyflavone	251 nm

2.2. Others Auxochromes

2.2.1. Glycosidic Substituent

In our study we synthesised some glycosidic derivatives from aglycones. Thus, we compared the absorption of the hydroxylated and the glycosylated flavones (Scheme 62).





82 Pratol R_1, R_2 = H and R_3 = OCH_3 **158** Pratol glucosyl R_1, R_2 = H and R_3 = OCH_3
119 Luteolin R_1, R_2, R_3 = OH **166** Luteolin glucosyl R_1, R_2, R_3 = OH

Scheme 62: Structure of glycosidic flavones and their precursors

We introduce the glucosyl group to enhance the solubility of the flavonoids in hydrophilic medium. We attempt to observe no effect on the absorption spectra induced from, because glucose itself has no UV activity.

2.2.1.1. Band (I)

The Figure 12 shows clearly the absence of effect on the wavelength for a glucosyl group. In the cases of Luteolin and Pratol, the introduction of a glucosyl rest on any flavonoid presents no effect (neither bathochromic nor hypsochromic). We can observe a hypochromic* effect on the absorption intensity, which is not reliable because it is more a question of purity of the compounds that decrease the absorption intensity.

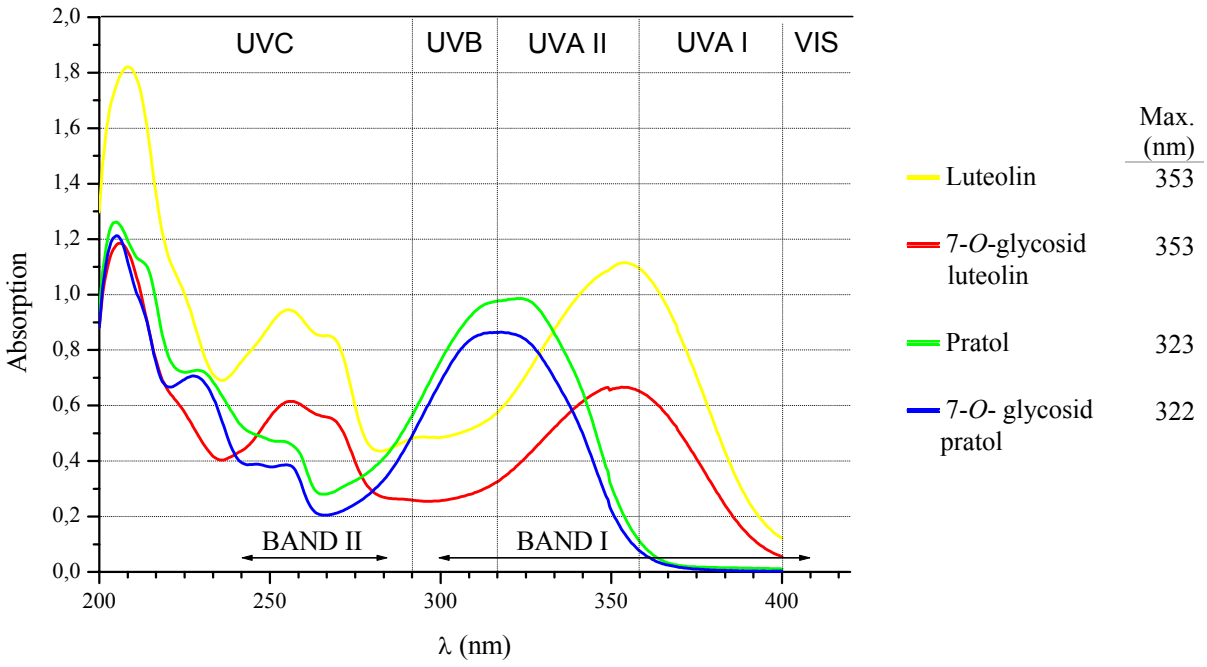


Figure 12: Substitution dependence of the absorption spectra of the hydroxylated and glycosylated flavones in isopropanol. Conc. 10 mM

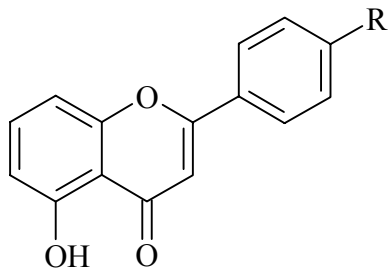
2.2.1.2. Band (II)

The same remark as in the paragraph Band (I) can be set here. The glucosyl group has no effect on the wavelength or on the absorption intensity.

* Hypochromic effect: an effect leading to decreased absorption intensity

2.2.2. Chloro- and aminosubstitutents

We compared here the 5-hydroxyflavone derivatives with a substituted B-ring by a: Hydroxyl, chloro and amino group at the 4′-position.



75 R = H
108 R = OH
128 R = Cl
130 R = NH_2

Scheme 63: Structure of 5-hydroxyflavone derivatives

2.2.2.1. Band (I)

We consider the 5-hydroxyflavone and introduce different auxochromes at the 4′-position and compare their absorption spectra. The Figure 13 points out the ineffectiveness of the chloride. The spectra of the 5-hydroxyflavone and the 4′-chloro-5-hydroxyflavone superpose themselves and have the same characteristics. The difference of one nanometer between the maxima of absorption is negligible.

The spectra of the hydroxy- and amino derivatives present a bathochromic effect, and more intense for the amino-substituted flavone with almost 50 nm towards the longer wavelength. Nearby this bathochromic effect, an important hyperchromic* effect on the absorption intensity is observed. Again the 4′-amino-5-hydroxyflavone presents the biggest effect with a gain of 325% of intensity, while the 4′,5-dihydroxyflavone gains 175% of absorption. The lone pair of the nitrogen atom of the amino group is more conjugated with the π-bond system of the benzene ring than the hydroxyl group, which could explain the fore mentioned observations.

* Hyperchromic effect: an effect leading to increased absorption intensity

The shoulder present at 300 nm in the spectra of 5-hydroxyflavone and 4′-chloro-5-hydroxyflavone disappears in the spectra of the other derivatives.

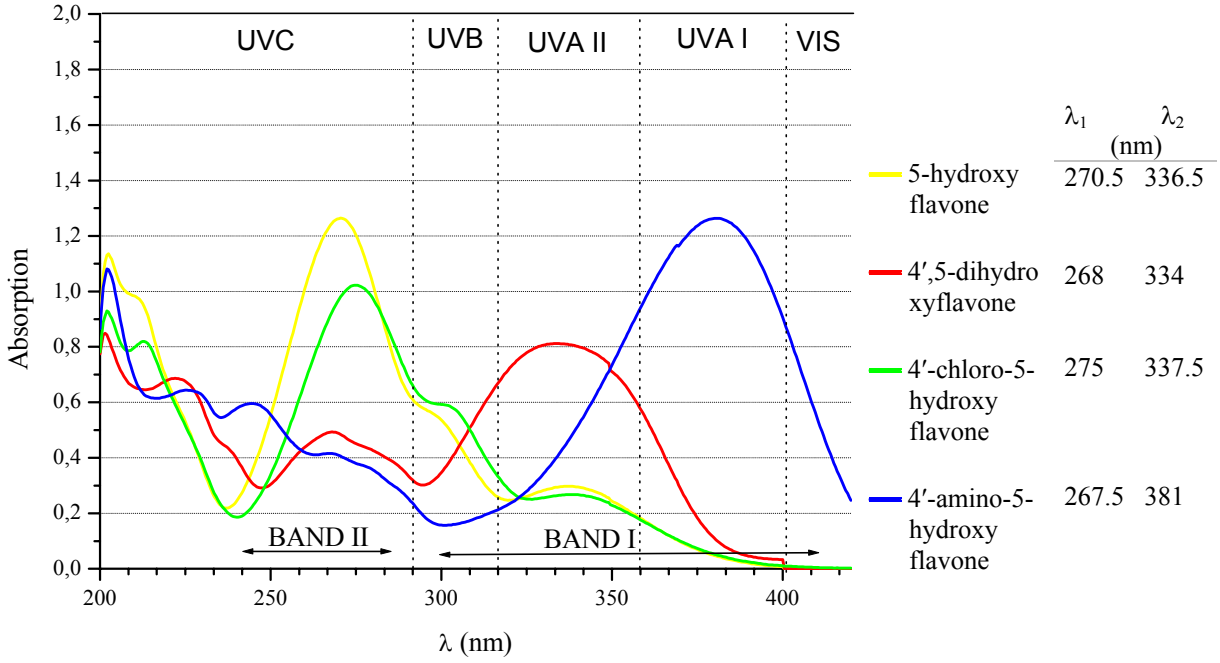


Figure 13: Substitution dependence of the absorption spectra of the 4′-substituted B-ring flavone derivatives in isopropanol. Conc. 10 mM

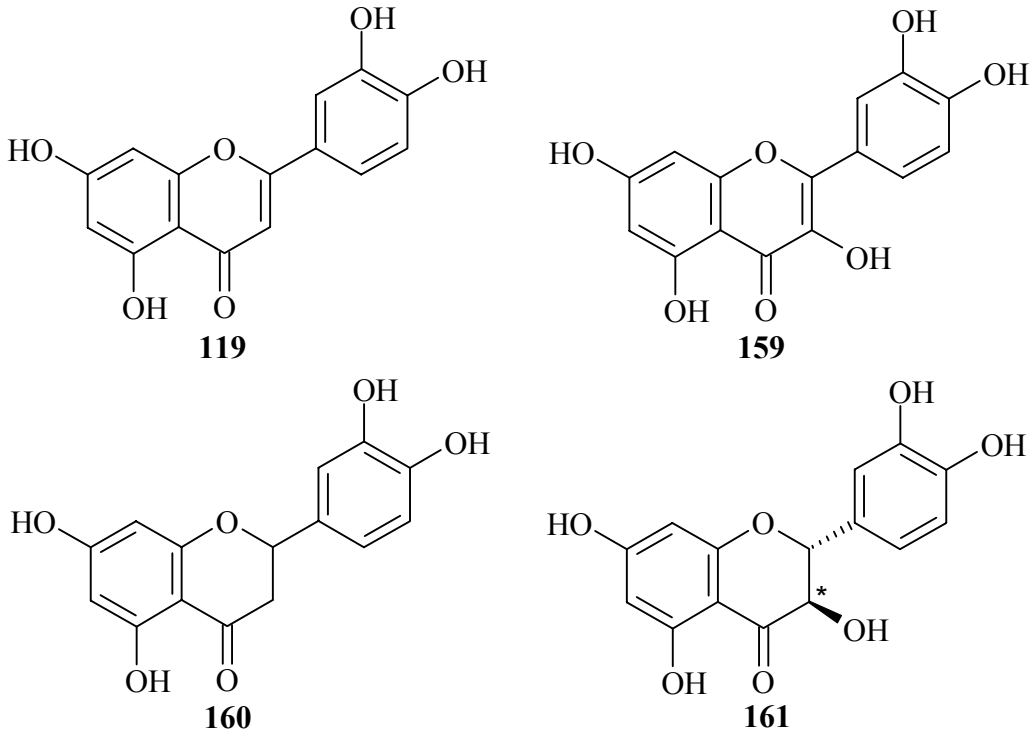
2.2.2.2. Band (II)

The Band (II) shows less intensive effects on the absorption spectra. The main effect to be observed is a hypochromic effect. While the 5-hydroxyflavone and the 4′-chloro-5-hydroxyflavone absorb intensively at about 270 nm, the 4′,5-dihydroxyflavone and 4′-amino-5-hydroxyflavone absorb at half this intensity. Neither bathochromic nor hypsochromic effect can be observed for this Band.

2.3. Flavonol, Flavanone, Flavanonol

After the study of Structure-UV activity Relationship of the flavones, we can see the influence of the structure of the C-ring by studying the typical examples of the corresponding families of flavonoids: flavone, flavonol, flavanones and flavanonol. These families of flavonoids differ in their structure by the oxidation pattern at the 3-position and the presence

or absence of a C-2/C-3 double bond. Thus, Luteolin (**119**), Quercetin (**159**), Eriodictyol (**160**) and Taxifolin (**161**) were selected (Scheme 64).



Scheme 64: Chemical Structure of Luteolin (**119**), Quercetin (**159**), Eriodyctiol (**160**) and Taxifolin (**161**).

2.3.1. Band (I)

A first look at the spectra (Figure 14) shows that the Luteolin and the Quercetin absorb in the UV-A range, whereas the Taxifolin and Eriodictyol absorb in the UV-B range. It is not surprising that breaking the conjugation in the middle of the structure of the flavonoid, can imply an important hypsochromic effect. This double bond causes two effects on the absorption spectra:

- ❖ On one hand, the maxima of the absorption are shifted between 40-20 nm towards the shorter wavelength.
- ❖ On second hand, an important hypochromic effect is observable: the intensity of the absorption decreases from 1.1 to 0.13 and from 0.71 to 0.10.

When we consider the Luteolin and introduce a hydroxyl group at the 3-position to get the Quercetin, the hydroxyl group causes a bathochromic effect (a gain of 20 nm in the long

wavelength) and a hypochromic effect (loss of 36% of the absorption intensity). But in the case of the Eriodictyol and Taxifolin, the introduction of the hydroxyl group has no incidence on the spectrum.

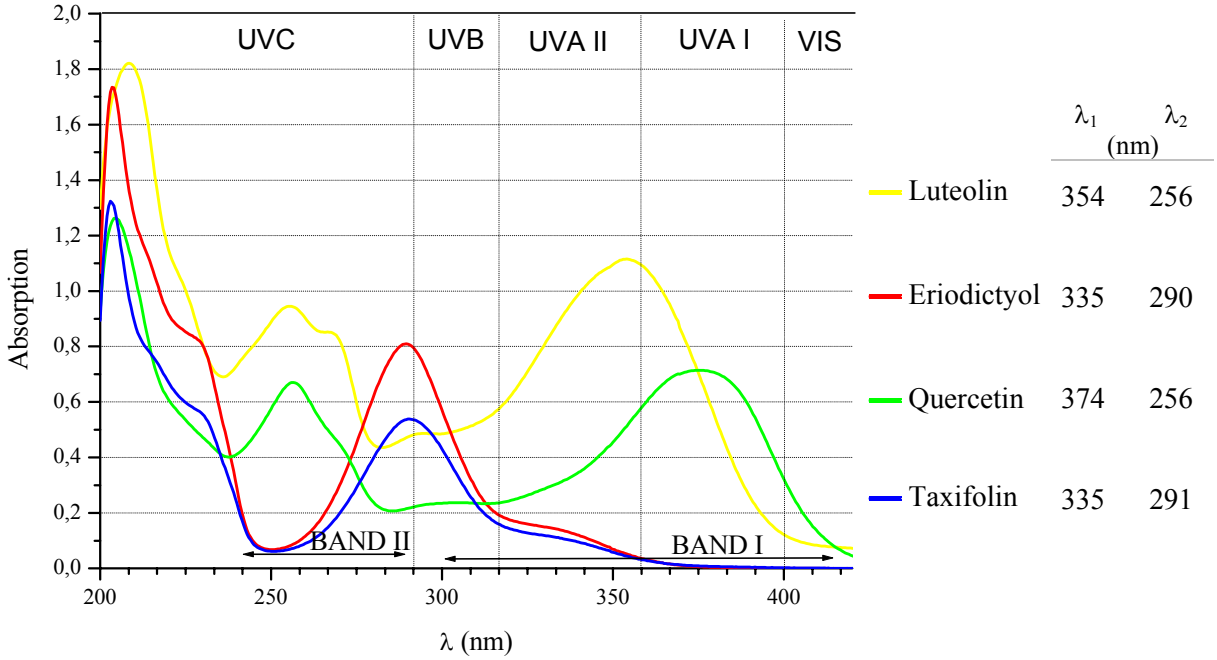


Figure 14: Substitution influence on the absorption spectra of various flavonoids

We can say that the double bond between the C-2 and C-3 is like an “electronic bridge” between the A- and B-rings, which allows the transfer of electrons between the rings B and C, and implies a bathochromic effect for the UV-absorption. Finally, the introduction of a hydroxyl group enhances the bathochromic effect only in presence of the double bond.

2.3.2. Band (II)

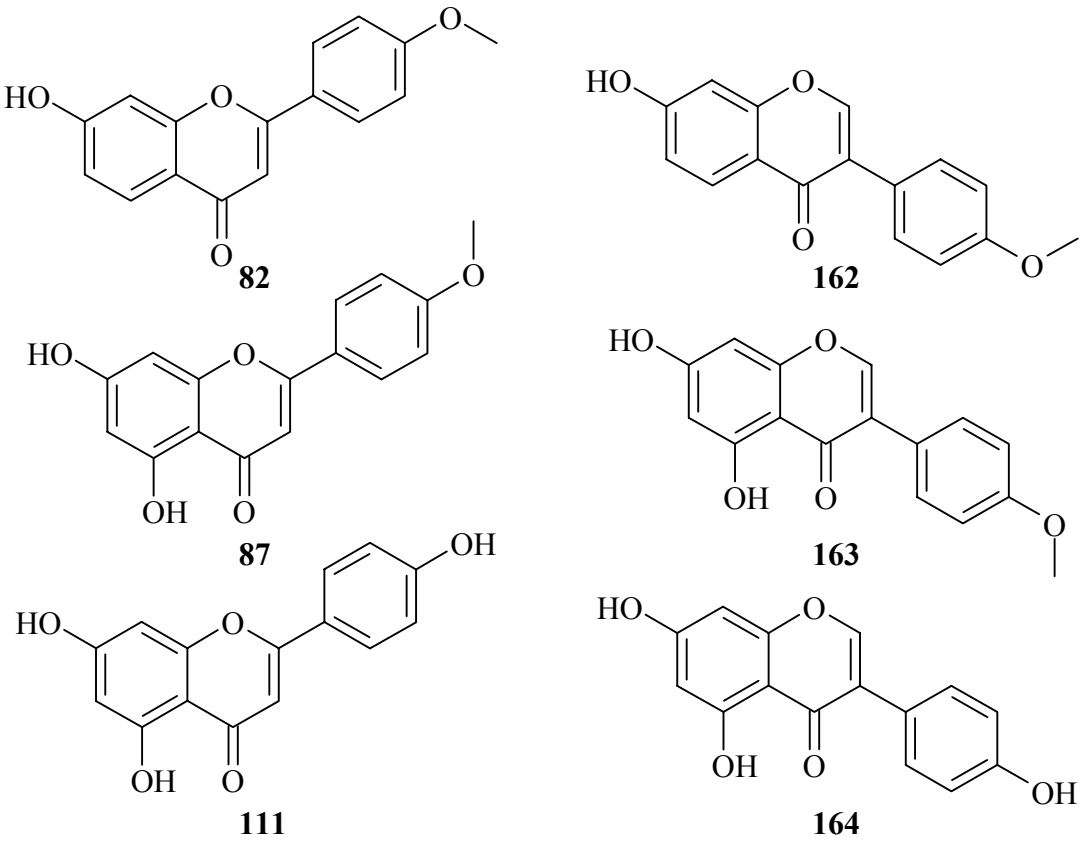
In opposition to the effect observed in the Band I, the suppression of the double bond between C-2 and C-3 in Luteolin and Quercetin causes a bathochromic effect, accompanied with a small hypochromic effect. In this case it deals with a shift of almost 40 nm of the absorption maxima that was not so intense in the Band (I).

The introduction of the hydroxyl group at the 3-position does affect the absorption. In both cases (Luteolin into Quercetin and Eriodictyol into Taxifolin) we can observe a

hypochromic effect with a loss of one third of the absorption at $\lambda_{2\,max}$, but we notice the absence of any hypsochromic or bathochromic effect.

2.4. Isoflavones

The isoflavones are structural isomers of the flavones (Scheme 65). A simple exchange between the substituents at the 2-position with those at the 3-position could explain further structural properties of the flavonoids.



Scheme 65: Structure of Pratol (**82**), Acacetin (**87**), Apigenin (**111**), Formononetin (**162**), Biochanin A (**163**) and Genistein (**164**).

2.4.1. Band (I)

The inversion between the substituents at the 2- and 3-position causes a hypochromic effect on the absorption intensity. While the Flavones absorb in the UVB and UVA II-ranges, the isoflavones present a weak absorption intensity in the same area. We can notice a small hypsochromic effect on the maxima of the absorption of flavone derivatives, but the determination of the maxima of isoflavones in band (I) can be discussed because the spectra

do not present any maxima but a decreasing shoulder (Figure 15).

The conclusions we obtained from the study of the flavone structure changes (position of the hydroxyl group, number of hydroxyl group, methylation of a hydroxyl group on the B-ring) are still valuable in the case of isoflavones. The only difference is the introduction of the second hydroxyl group on the A-ring at the 5-position causes a bathochromic effect with a shift of 29 nm, while the demethylation of the methoxy group on the B-ring presents no effect on the Band (I).

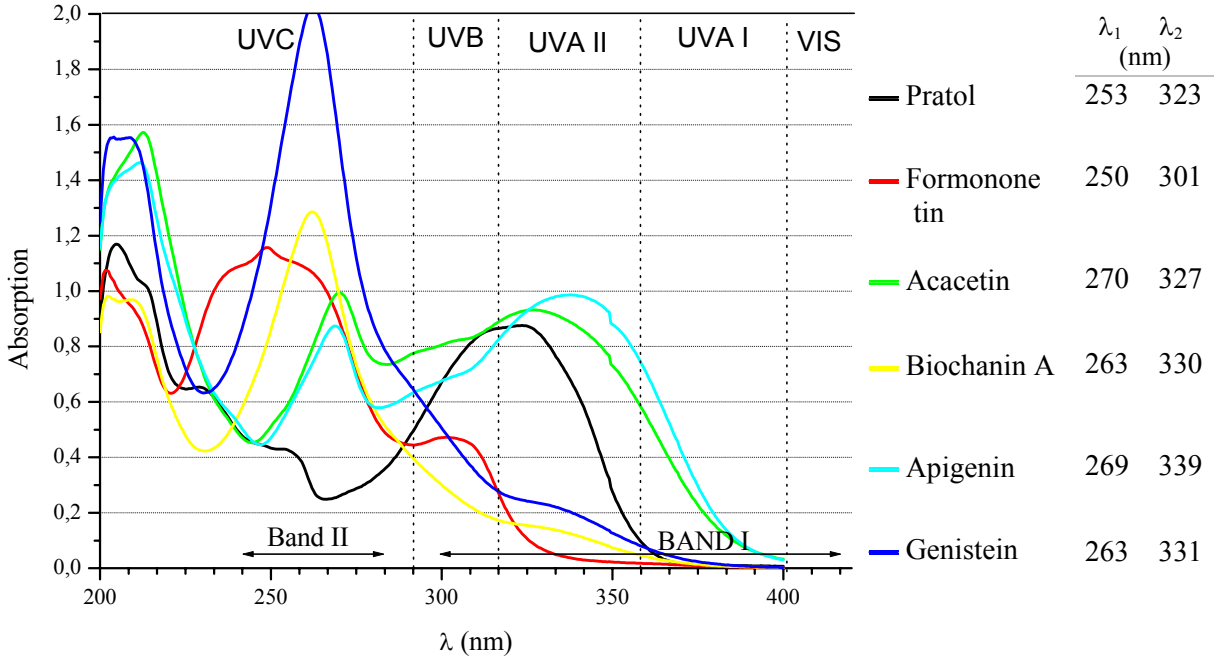


Figure 15: Structure influence on the absorption spectra of flavones and isoflavones in isopropanol. Conc. 10 mM

2.4.2. Band (II)

In opposition to the Band (I), we can notice that the Band (II) presents a hypsochromic effect, with a shift of –7 nm towards the shorter wavelengths for the comparison of flavones in relation with isoflavones. This effect is accompanied with an important hyperchromic effect. A gain of absorption in the UVC range is clearly observed. Formononetin (**162**) and Genistein (**164**) are two good examples: the absorption rises from 0.42 to 1.15 (+174%) and

from 0.87 to 2.03 (+133%) respectively.

As we remarked in the above paragraph, the introduction of a second hydroxyl group presents a bathochromic effect of 13 nm. Again the substitution of the B-ring of isoflavones does not affect the absorption spectra.

3. Conclusion

We can formulate the following conclusions for the structure-UV activity relationship of flavonoids:

- Strong influence of one hydroxyl group at the 5- or 8-positions
- Strong influence of two groups at the 3′- and 4′-positions
- Strong influence of the amino group at the 4′ position
- Strong influence of the double bond between C-2 and C-3
- Strong influence of the hydroxyl group at the 3-position only in presence of the double bond between C-2 and C-3
- Weak influence of one hydroxyl group at the 7-position
- Middle influence of two group at the 5- and 7-positions

Having in mind these informations, we would be able to build[132] a flavonoid with a desired UV-spectrum (Figure 16). We suggest a combination of three different flavonoids that absorb in UVB-, UVA II- and UVA I- ranges: 7-hydroxyflavone (**73**) (308 nm, ε 18700), 6,3′,4′-trihydroxyflavone (**115**) (339 nm, ε 27600) and 5-hydroxy-4′-aminoflavone (**130**) (381 nm, ε 32000). The last one could also be replaced by Quercetin (**159**) (374 nm, ε 21598).

We can quote three other flavones, which absorb beyond one UV domain: it is the Pratol (**82**) (323 nm, ε 23490) as filter between UVB- and UVA II- ranges, 5,7-dihydroxy-4′-

methoxyflavone (**87**) (327 nm, ε 26495) as UVB and UVA II filter, and Luteolin (**119**) (343 nm, ε 28625) as filter between UVA II- and UVA I- range.

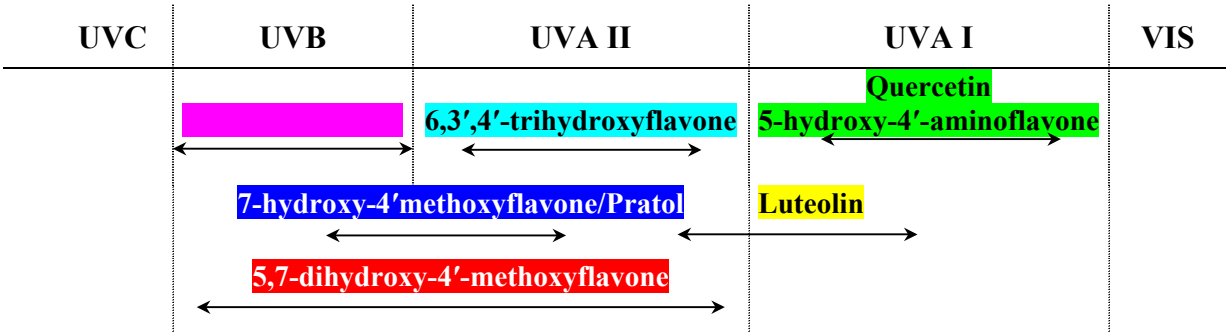


Figure 16: Combination of different flavonoids to cover the entire UV-ranges

Chapter 6: Structure Antioxidant Activity Relationship

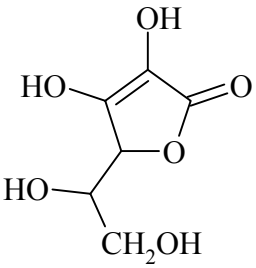
1. Introduction

Oxidation is the transfer of electrons from one atom to another and represents an essential part of aerobic life and our metabolism, since oxygen is the ultimate electron acceptor in the electron flow system that produces energy in the form of ATP.[133] However, problems may arise when the electron flow becomes uncoupled (transfer of unpaired single electrons), generating free radicals. Examples of oxygen-centred free radicals, known as reactive oxygen species (ROS), include superoxide ($O_2^{\bullet -}$), peroxyl ($ROO^{\bullet}$), alkoxyl ($RO^{\bullet}$), hydroxyl ($HO^{\bullet}$), and nitric oxide ($NO^{\bullet}$). The hydroxyl (half life of 10^{-9} s) and the alkoxyl (half life of seconds) free radicals are very reactive and rapidly attack the molecules in nearby cells, and probably the damage caused by them is unavoidable and is dealt with by repair processes. In addition to these ROS radicals, in living organisms there are other ROS nonradicals, such as the singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), and hypochlorous acid (HOCl).

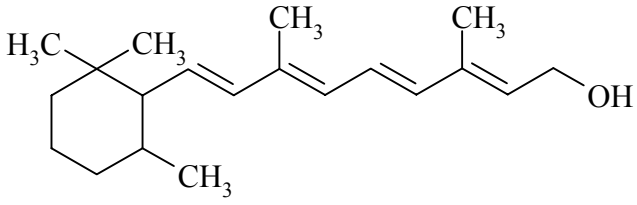
It is accepted that ROS play different roles in vivo. Some are positive and are related to their involvement in energy production, regulation of cell growth, and synthesis of biologically important compounds.[134] However, ROS may be very damaging, since they can attack lipids in cell membranes, proteins in tissues or enzymes, carbohydrates, and DNA, to induce oxidations, which cause membrane damage, protein modification (including enzymes), and DNA damage. Humans have evolved with antioxidant systems to protect against free radicals. These systems include some antioxidants produced by the body (endogenous) and other obtained from the diet (exogenous). The various endogenous defenses (enzymatic and nonenzymatic) are complementary to each other, since they act against different species at different cellular compartments. However, despite these *defense antioxidants* (able either to suppress free radical formation and chain initiation or to scavenge free radical and chain propagation), some ROS still escape to cause damage. Thus, the body antioxidant system is

provided also by *repair antioxidants* (able to repair damage, and based on proteases, lipases, transferases, and DNA repair enzymes).[135]

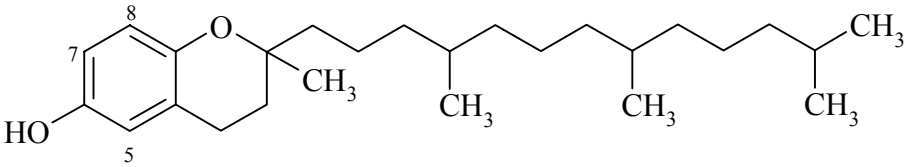
Owing to the incomplete efficiency of our endogenous defense systems and the existence of some physiopathological situations (cigarette smoke, air pollutants, UV radiation, high polyunsaturated fatty acid diet, inflammation, ischemia/reperfusion, etc.) in which ROS are produced in excess and at the wrong time and place, antioxidants are needed for diminishing the cumulative effects of oxidative damage over the life span.[136,137] Well established antioxidants derived from the diet are vitamins C, A and E (Scheme 66), which have been studied intensively.[138] Beside these antioxidant vitamins, other substances in plants might account for at least part of the health benefits associated with vegetable and fruit consumption. Over the past decade evidence has been accumulated that plant polyphenols are an important class of defense antioxidants, and are widespread virtually in all plant food, and include phenols, phenolic acids, flavonoids, tannins, and lignans.



Vitamin C
(ascorbic acid)



Vitamin A



Vitamin E
(Tocopherol)

Scheme 66: Chemical structure of vitamins C, A, and E

2. Antioxidant Activity

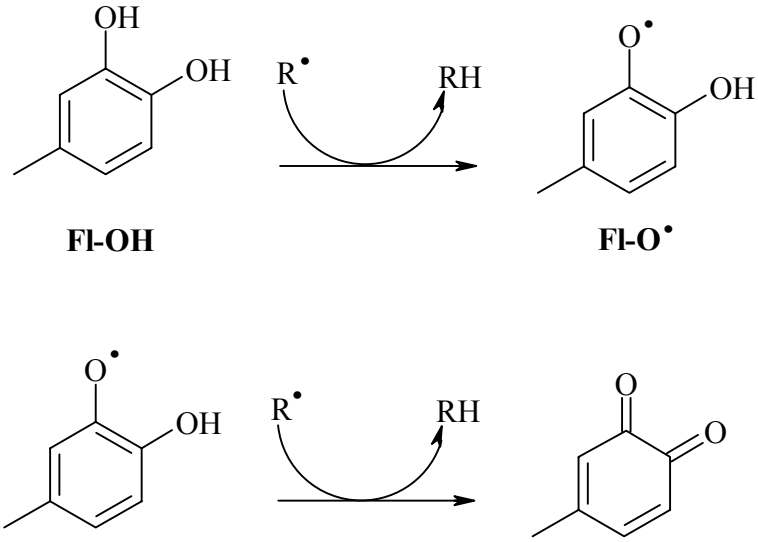
2.1. Definition of the antioxidant activity

The chemical activities of polyphenols in terms of their reducing properties as hydrogen- or electron-donating agents predicts their potentials for action as free radical scavengers (antioxidants). The activity of an antioxidant is determined by:

- Its reactivity as hydrogen- or electron-donating agent (which relates to its reduction potential).
- The fate of the resulting antioxidant-derived radical, which is governed by its ability to stabilize and delocalise the unpaired electron.[139]
- Its reactivity with other antioxidants.
- The transition metal-chelating potential.

2.2. Mechanisms of the antioxidant action

The mechanism of the protective action of the flavonoids is a subject of considerable debate. As polyphenolic compounds, flavonoids have the ability to act as antioxidants by a free radical scavenging mechanism (Scheme 67) with the formation of less flavonoid phenoxyl radicals (FlO$^{\bullet}$).



Scheme 67: Scavenging of ROS (R$^{\bullet}$) by flavonoids

Due to their lower redox potential ($0.23 < E7 < 0.75$ V)[140] flavonoids are thermodynamically able to reduce highly oxidazing free radical with redox potential in the range 2.13-1.0 V,[141] such as superoxide, peroxyl, alkoxyl, and hydroxyl radicals by hydrogen atom donation:

$$\text{Fl-OH} + \text{R}^{\bullet} \rightarrow \text{Fl-O}^{\bullet} + \text{RH} \qquad (1)$$

where $R^{\bullet}$ represents superoxide anion, peroxyl, alkoxyl, and hydroxyl radicals. The aroxyl radical ($FlO^{\bullet}$) may react with a second radical entity, acquiring a stable quinone structure (Scheme 67). The aroxyl radicals could interact with oxygen, generating quinines and superoxide anion, rather than terminating chain reactions. The last reaction may take place in the presence of high levels of transient metal ions and is responsible for the undesired prooxidant effect of flavonoids.[142]

On the hand, through their known ability to chelate transition metals, these compounds may inactivate iron ions through complexation, thereby suppressing the superoxide driven Fenton Reaction (2 and 3), which is currently believed to be the most important route to active oxygen species.

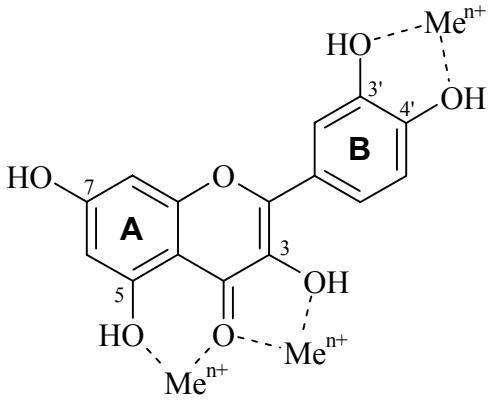
$$O_2^{\bullet -} + \text{Fe(III)} \rightarrow O_2 + \text{Fe(II)} \qquad (2)$$

$$\text{Fe(II)} + H_2O_2 \rightarrow \text{Fe(III)} + HO^{\bullet} + HO^{-} \qquad (3)$$

Despite the early realization by researchers that the structures of these compounds allow them to form heavy metal complexes (Scheme 68), metal chelation has generally been regarded to play a minor role in the antioxidant activity of flavonoids. Nevertheless, it has to be remembered that these metal ions are essential for many physiological functions, as constituents of hemoproteins and cofactors of different enzymes, including those involved (iron for catalase, copper for ceruloplasmin and *Cu,Zn*-superoxide dismutase) in the antioxidant defense.[143]

The proposed binding sites for the trace metals to flavonoids are the catechol moiety in the B-ring, the 3-hydroxyl, 4-oxo groups in the heterocyclic ring, and the 4-oxo, 5-hydroxyl

groups between the heterocyclic ring and the A-ring (Scheme 68).

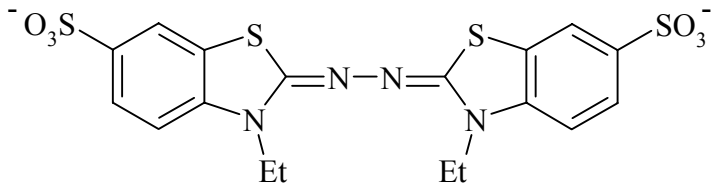


Scheme 68: Binding sites for trace metals

2.3. Methods of the *in vitro* antioxidant activity

2.3.1. Trolox Equivalent Antioxidant Capacity (TEAC)

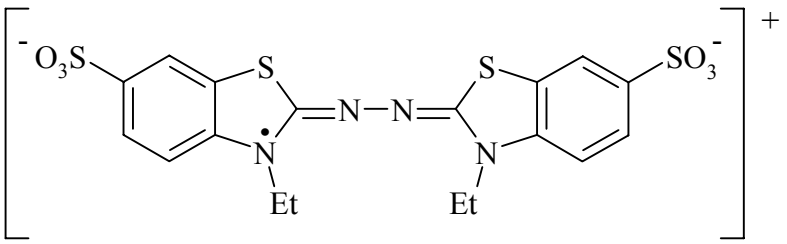
A valuable assay that allows for the determination of the hierarchy of radical-scavenging ability of flavonoids (and related phenolic acids) is based on the ability of an antioxidant to scavenge (at pH 7.4) a preformed radical cation chromophore of 2,2′-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS$^{\bullet+}$) (Scheme 69) in relation to that of



ABTS

(2,2'-azinobis-(3-ethylbenzthiazoline-6-sulphonic acid))

Potassium persulfate | - e$^-$



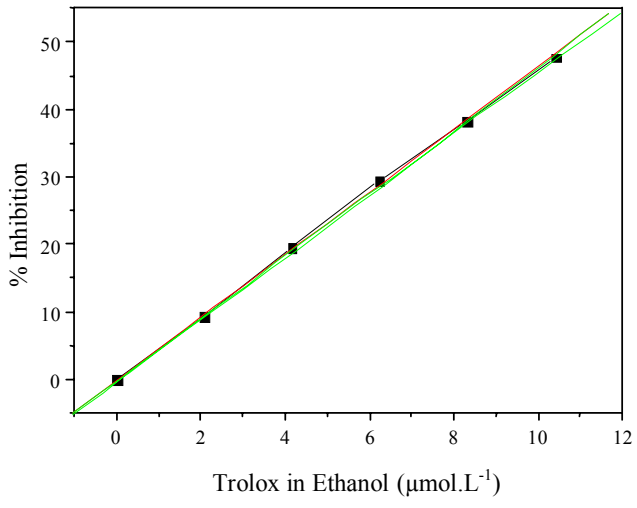
ABTS $^{\bullet+}$

Scheme 69: Formation of the ABTS radical cation

6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox or α-tocopherol), an aqueous soluble vitamin E analogue.

This method involves the formation of the radical cation $ABTS^{\bullet+}$ through oxidation by potassium persulfate in aqueous solution. The radical cation presents three absorption maxima at 645 nm, 734 nm, and 815 nm. The antioxidant under investigation is exposed to the radical cation $ABTS^{\bullet+}$ for a defined time period, and leads to the reduction to ATBS. The yield of the reduction depends on the activity and the concentration of the antioxidant as well as the duration of the reaction. A spectrophotometric measurement of the extent of the quenched radical shows the reduction. The percentage of the reduced ABTS is plotted as a function of concentration and time, relatively to that of Trolox. The Trolox equivalent antioxidant capacity (TEAC) is defined as the concentration of Trolox with the same antioxidant capacity (concentration necessary to cause the same decrease of $ABTS^{\bullet+}$ at 734 nm) as a 1 mM concentration of the antioxidant under investigation.

The extinction values are measured after 6 minutes. The percentage of the reduced ABTS is plotted in relation to the concentration of the studied substance and of Trolox. The TEAC value is the ratio between the slope of the Trolox function and the slope of the substance function. The examples of the measurement of the antioxidant activity of some flavonoids in comparison to Trolox are shown below.



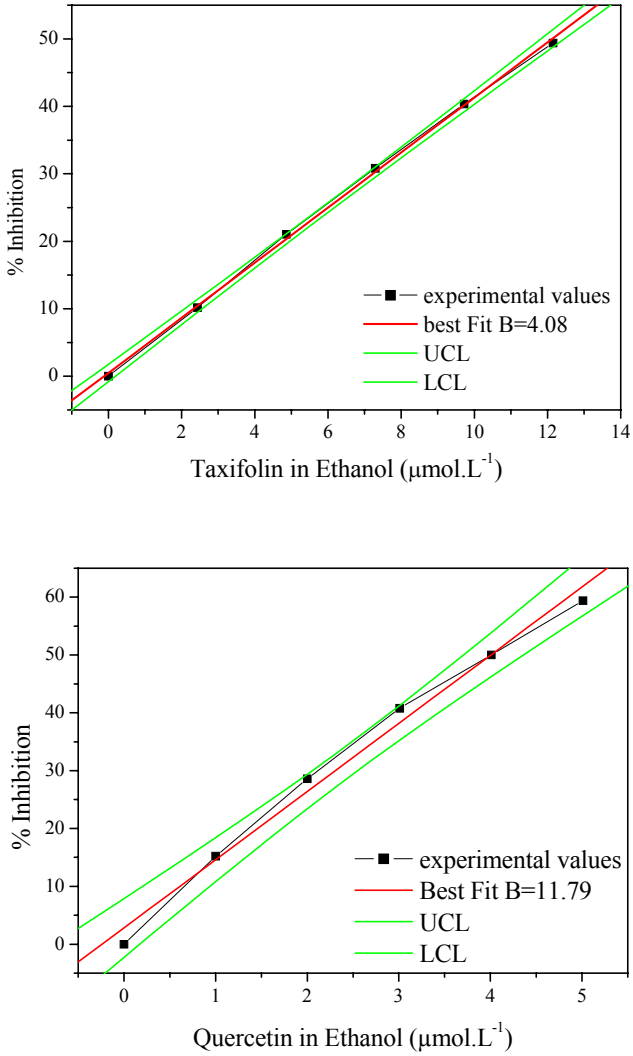
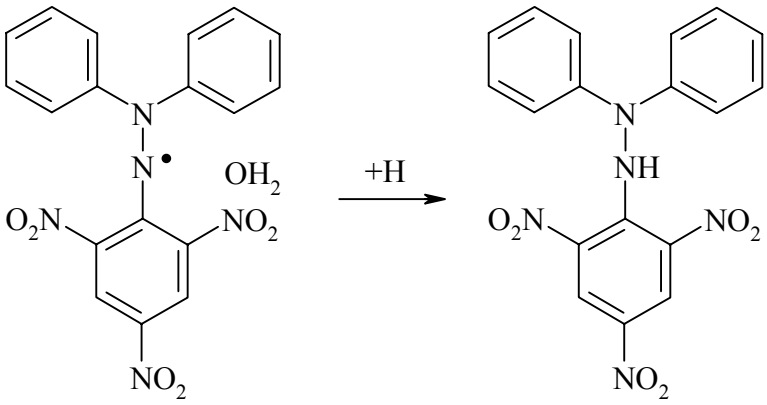


Figure 17: measurement of the antioxidant activity of some flavonoids

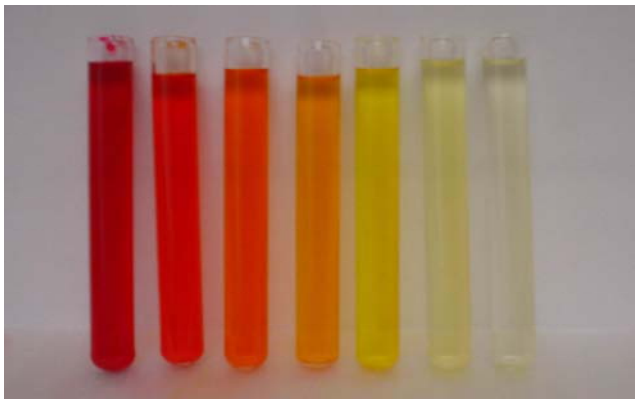
2.3.2. DPPH assay

This assay is used to screen the antioxidative or radical scavenging potential of a substance or an extract. The kinetic behaviour of flavonoids as free radical scavengers was studied using the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•), which is a relative stable paramagnetic free radical that accepts electrons of hydrogen radical to become a stable diamagnetic molecule (Scheme 70). Another characteristic of the 2,2-diphenyl-1-picrylhydrazyl hydrate is its colour, violet, which is modified to yellow in presence of a free radical quencher, by appearance of the 2,2-diphenyl-1-picrylhydrazine (Picture 4).



DPPH• DPPH-H

Scheme 70: 2,2-diphenyl-1-picrylhydrazyl hydrate and its reduced form



Picture 4: Decolourisation of the DPPH• into DPPH-H

Monitoring the decrease of the optical density in its characteristic wavelength during the reaction follows the reduction of DPPH• in an alcohol solution, because in its radical form DPPH• absorbs at 515 nm, but upon the reduction by an antioxidant or radical scavenger (AH) the absorption disappears.

$$DPPH^{\bullet} + AH \rightarrow DPPH\text{-}H + A^{\bullet} \quad (4)$$

After addition of different standard concentrations of the antioxidant under investigation to DPPH• (0.025 $g.L^{-1}$), the percentage of remaining DPPH• was determined at different times (1 second, 2 minutes, 10 minutes, and then every 10 minutes, until the absorption intensity is constant) from the absorbances at 515 nm. The percentage of remaining DPPH• against the standard concentration was plotted to obtain the amount of antioxidant necessary to decrease the initial DPPH• concentration to 50% (EC_{50}) (Figure 18).

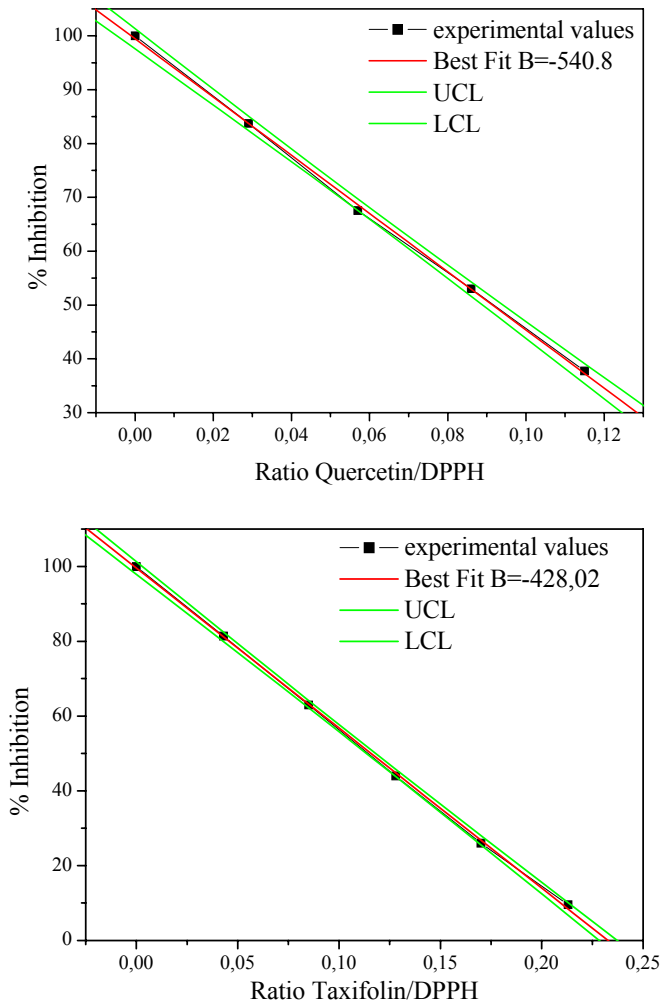
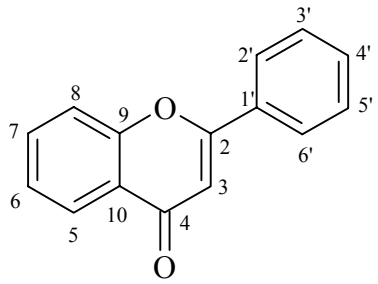


Figure 18: measurement of the antioxidant scavenging the DPPH•

The smaller this value is, the bigger is the activity against the radicals. This parameter, EC_{50}, is widely used to measure antioxidant power, but it does not take into account the reaction time because each antioxidant has a different behaviour: it can react slowly, medium or quickly[144] and it can take between 30 seconds to 12 hours to reach the steady state. Thus, the time needed to reach the steady state to the concentration corresponding at EC_{50} (T_{EC50}) is calculated to discriminate flavonoids with no significant difference between their EC_{50}, and antiradical efficiency (AE) is used as new parameter[145] to characterise the antioxidant compounds.

$$AE = \frac{1}{EC_{50}\, T_{EC50}} \qquad (5)$$

The bigger the AE value is, the bigger is the activity against the radicals.



	OH arrangement	OMe arrangement	TEAC	EC_{50}	T_{EC50}
24	-	-	< 0.01	*n.d.*	*n.d.*
73	7	-	< 0.01	*n.d.*	*n.d.*
74	6	-	*n.d.*[a]	*n.d.*[a]	*n.d.*[a]
75	5	-	< 0.01	*n.d.*	*n.d.*
76	7,8	-	0.98	0.21	600
77	6,7	-	1.72	0.11	600
78	5,7	-	0.01	*n.d.*	*n.d.*
79	5,6,7	-	2.29	0.10	360
80	-	4′	< 0.01	*n.d.*	*n.d.*
82	7	4′	*n.d.*[b]	*n.d.*[b]	*n.d.*[b]
83	6	4′	< 0.01	*n.d.*[a]	*n.d.*[a]
84	5	4′	< 0.02	*n.d.*[a,c]	*n.d.*[a,c]
87	5,7	4′	*n.d.*[b]	*n.d.*[b]	*n.d.*[b]
88	-	3′,4′	*n.d.*[b]	*n.d.*[b]	*n.d.*[b]
90	7	3′,4′	*n.d.*[b]	*n.d.*[b]	*n.d.*[b]
91	6	3′,4′	< 0.05	*n.d.*[a]	*n.d.*[a]
92	5	3′,4′	*n.d.*[b]	*n.d.*[b]	*n.d.*[b]
93	7,8	3′,4′	1.05	0.12	180
95	5,7	3′,4′	*n.d.*[a]	*n.d.*[a]	*n.d.*[a]
96	-	3′,4′,5′	0.89	*n.d.*[d]	*n.d.*[d]
101	7,8	3′,4′,5′	*n.d.*[b]	*n.d.*[b]	*n.d.*[b]
102	6,7	3′,4′,5′	1.50	0.15	800
103	5,7	3′,4′,5′	*n.d.*[b]	*n.d.*[b]	*n.d.*[b]
106	7,4′	-	< 0.02	*n.d.*[b]	*n.d.*[b]
107	6,4′	-	< 0.05	*n.d.*[a]	*n.d.*[a]
108	5,4′	-	0.01	*n.d.*	*n.d.*
109	7,8,4′	-	0.92	0.11	600
111	5,7,4′	-	*n.d.*[b]	*n.d.*[b]	*n.d.*[b]
114	7,3′,4′	-	1.00	0.16	600
115	6,3′,4′	-	1.06	0.12	180
116	5,3′,4′	-	*n.d.*[b,e]	*n.d.*[b,e]	*n.d.*[b,e]
117	7,8,3′,4′	-	1.41	0.09	600
119	5,7,3′,4′	-	0.89	0.13	600
128	5	-	0.01	*n.d.*	*n.d.*
130	5	-	0.03	*n.d.*	*n.d.*

n.d.: not determined; [a] not linear reaction; [b] not or partially soluble; [c] not pure; [d] shows a weak activity in DPPH assay and very bad solubility; [e] the suspension shows a strong activity.

Table 11: Antioxidant activity (TEAC (mM), efficient concentration EC_{50}, and time of efficient concentration T_{EC50} (min)) of synthesized flavones relative to Trolox.

3. Structure-Antioxidant Activity Relationship

Flavonoids have been the study of many SAR for their antioxidant,[146,147,148,149,150] radioprotective,[151] radical scavenging[152,153] or prooxidant capacities.[154] We tried to show in this paragraph some new elements of the antioxidant character of flavonoids. The examined flavones showed different solubility patterns in the experiment media, and these differences in solubility may influence results of the tests. For this reason we gave here the results of the antioxidant activities that were established for the flavones presenting a complete solubility in the experimental media (Table 11).

3.1. TEAC

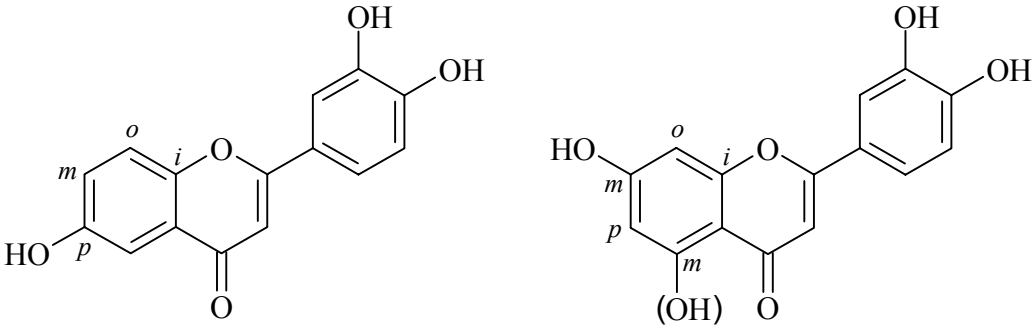
Even though none of the analysed compounds were as good as Quercetin, it is worthy to note that only four products have a TEAC value clearly greater than 1.0. For this reason, we restricted our study to the flavones presenting significant TEAC values (Table 12).

	TEAC		**TEAC**
Quercetin (**159**)	2.78	7,8-dihydroxy-3′,4′-dimethoxyflavone (**93**)	1.05
Bacalein (**79**)	2.29	7,3′,4′-trihydroxyflavone (**114**)	1.00
6,7-dihydroxyflavone (**77**)	1.72	7,8-dihydroxyflavone (**76**)	0.98
6,7-dihydroxy-3′,4′,5′-trimethoxyflavone (**102**)	1.50	7,8,4′-trihydroxyflavone (**109**)	0.92
7,8,3′,4′-tetrahydroxyflavone (**117**)	1.41	Luteolin (**119**)	0.89
6,3′,4′-trihydroxyflavone (**115**)	1.06	Taxifolin (**161**)	0.87

Table 12: Total Antioxidant Activities (mM) relative to Trolox of the significant flavonoids

3.1.1. Influence of the hydroxyl group position on the A-ring

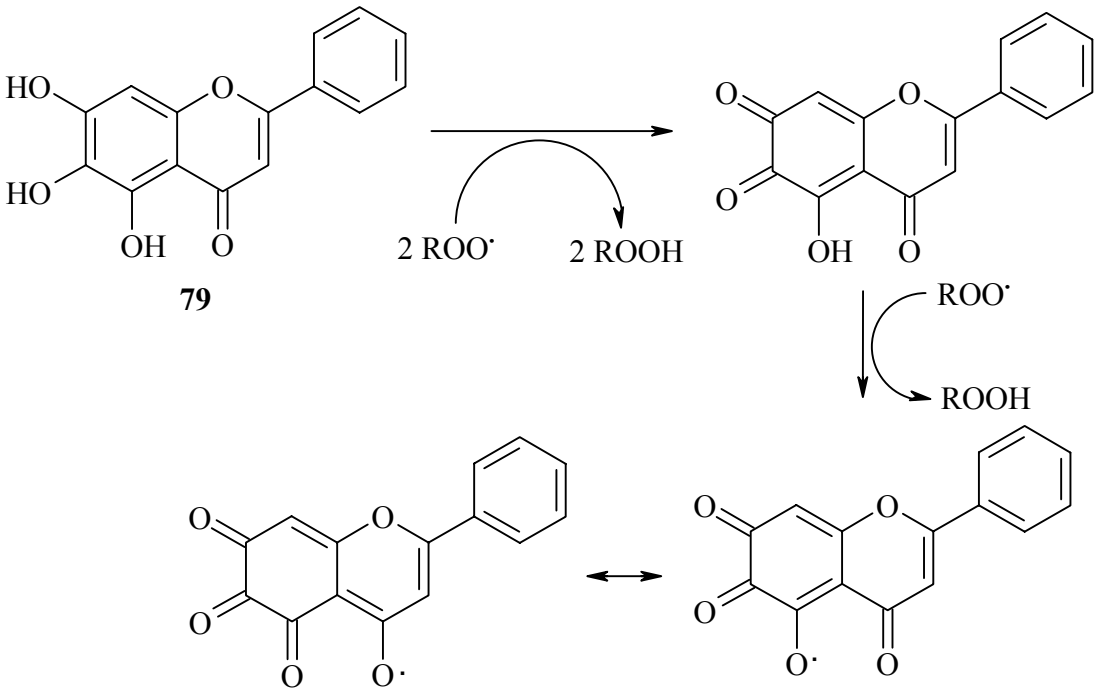
In the case of flavones **115**, **114** and **119,** the 6,3′,4′-trihydroxy combination (TEAC = 1.06) is a bit better than the 7,3′,4′-trihydroxy combination (TEAC = 1.00) and clearly better to 5,7,3′,4′-tetrahydroxy combination (e.g. Luteolin TEAC = 0.89). The 6-position (in *para* of the oxygen atom of the pyrone ring) is more efficient to scavenge a free radical than the 7-position and the 5-, and 7-positions (both are in *meta* of the oxygen atom of the pyrone ring) (Scheme 71).



115 **114** or **119**

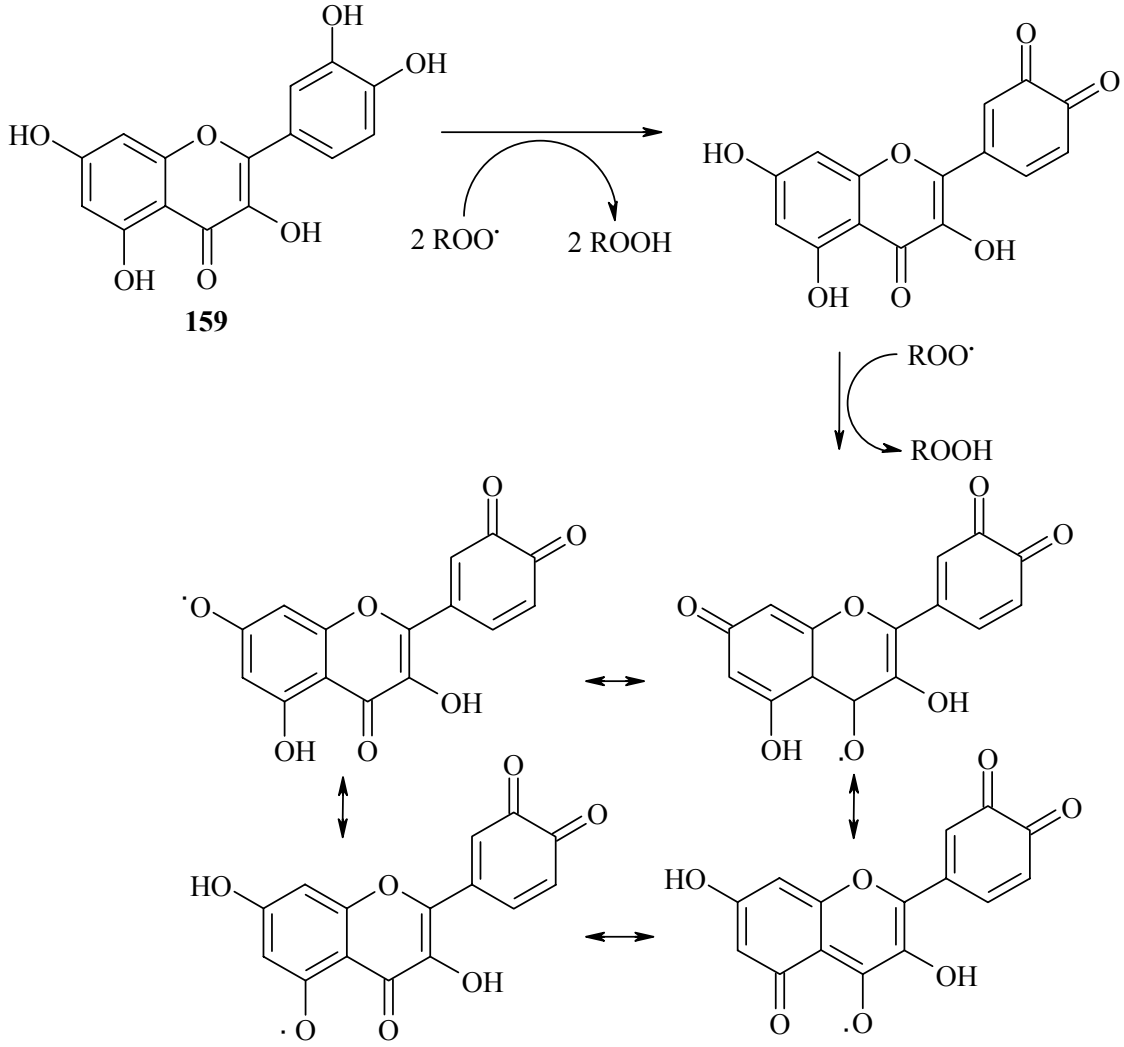
Scheme 71: position of the hydroxyl groups in flavones **115**, **114** and **119**

If we consider the Bacalein (**79**) with its 3 hydroxyl groups at 5-, 6-, and 7-positions on the A-ring, the 6,7-dihydroxyflavone (**77**), the 6,7-dihydroxy-3′,4′,5′-trimethoxyflavone (**102**), and the 7,8,3′,4′-tetrahydroxyflavone (**117**) with two hydroxyl groups on the A-ring, we can noticed that the presence of three hydroxyl groups (at 5-, 6-, and 7-positions) is up to now the best possible combination on the A-ring. Moreover, in the case of the Bacalein, this combination of hydroxyl groups is the unique substitution pattern on the flavone skeleton, and manifests itself in a TEAC value very close to the one of Quercetin. Scheme 72 shows the mechanism of ROS scavenging of Bacalein (**79**). It can quench up to three ROS and get a triketone radical structure, which is stabilized by the mesomeric forms.



Scheme 72: Mechanism of ROS Scavenging of Bacalein (**79**)

If we observe the mechanism of ROS scavenging of the Quercetin (**159**) in Scheme 73, we can notice that Quercetin quenched three ROS but present more mesomeric forms than the triketone radical of Bacalein, and it could explain the values measured in the TEAC assay.

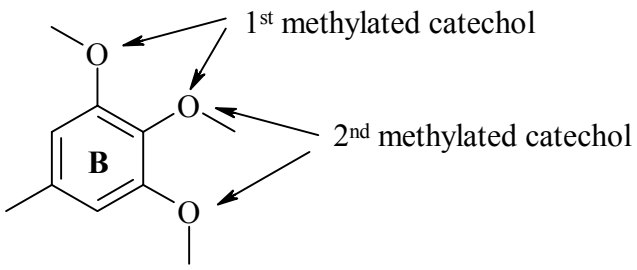


Scheme 73: Mechanism of ROS scavenging Quercetin (**159**)

As soon as the number of hydroxyl groups is limited to two (or one) on the A-ring, the antioxidant activity of the flavone decreases. Interestingly, the combination of a catechol, which is known to complex ions but also to be an optimal structure to stabilise radicals *via* a quinone structure, at the 6- and 7-positions (TEAC=1.72) appears to be better than the combination of a catechol at the 7- and 8-positions (TEAC=0.98) to perform a stabilisation of free radicals.

3.1.2. Influence of the substitutions on the B-ring

Considering the 7,8-dihydroxyflavone derivatives (**76**, **93**, **109** and **117**), we are in presence of four substitution patterns of the B-ring: 3′,4′-dihydroxy (TEAC 1.41), 3′,4′-dimethoxy (TEAC 1.05), 4′-hydroxyl (TEAC 0.92) and just protons (TEAC 0.98). The two last values are rather surprising because we would expect that the introduction of one hydroxyl group on the B-ring would confer to the molecule a better scavenging potential than a simple phenyl ring. At the same time, they are very close and certainly within the margin error. In the case of the 7,8,4′-trihydroxyflavone (**109**), if we introduce a second hydroxyl group at the 3′-position the formation of a catechol on the B-ring (**117**) shows an important rise of the TEAC value (from 0.92 to 1.41). These results are confirmed by those of the literature, and we can say that the 3′,4′-dihydroxy structure contributes about 30% to the antioxidant activity of the compound. The methylation at all of these crucial positions influences negatively the antioxidant activity of the flavones by a loss of 25% of the activity. At the same time, the presence of three methoxyl groups on the B-ring (flavone **102**) in comparison to flavone (**77**), shows an important decrease of the antioxidant activity with a loss of 13% of the activity. The explanation could be explained with the 3D structure. As we explained in chapter 4, three methoxyl groups on the B-ring present an "out of plane" conformation. Instead of considering the three *ortho* methoxyl groups as a "double fused methylated catechol" (Scheme 74), each methoxyl group, due to its conformation, seems to be unique.



Scheme 74: Definition of the "double fused catechol"

3.2. DPPH assay

The amount of antioxidant necessary to decrease by 50% the initial DPPH$^{\bullet}$ concentration (EC_{50}), the time needed to reach the steady state at EC_{50} concentration (T_{EC50}), and the antiradical efficiency (AE) were established among some flavones (Table 13).

Nr	Compound	EC_{50}	T_{EC50}	AE (x10^{-3})
77	6,7-dihydroxyflavone	0.11	600	15.15
76	7,8-dihydroxyflavone	0.21	600	7.94
79	5,6,7-trihydroxyflavone/ Bacalein	0.10	360	27.78
93	7,8-dihydroxy-3′,4′-dimethoxyflavone	0.12	180	46.30
94	6,7-dihydroxy-3′,4′,5′-trimethoxyflavone	0.15	800	8.33
109	7,8,4′-trihydroxyflavone	0.11	600	15.15
115	6,3′,4′-trihydroxyflavone	0.12	180	46.30
114	7,3′,4′-trihydroxyflavone	0.16	600	10.42
119	5,7,3′,4′-tetrahydroxyflavone/Luteolin	0.13	600	12.82
117	7,8,3′,4′-tetrahydroxyflavone	0.09	600	18.52
159	3,5,7,3′,4′-pentahydroxyflavone /Quercetin	0.089	600	18.72
161	3,5,7,3′,4′-pentahydroxyflavanone/Taxifolin	0.15	1200	5.53

Table 13: antioxidant activity of selected flavonoids *versus* DPPH cation radical

The results have shown that the order of AE (x10^{-3}) in the tested compounds was: 6,3′,4′-trihydroxyflavone, 7,8-dihydroxy-3′,4′-dimethoxyflavone (**46.30**) > Bacalein (**27.78**) > Quercetin (**18.72**) > 7,8,3′,4′-tetrahydroxyflavone (**18.52**) > 6,7-dihydroxyflavone, 7,8,4′-trihydroxyflavone (**15.15**) > Luteolin (**12.82**) > 7,3′,4′-trihydroxyflavone (**10.42**) > 6,7-dihydroxy-3′,4′,5′-trimethoxyflavone (**8.33**) > 7,8-dihydroxyflavone (**7.94**) > Taxifolin (**5.33**).

3.2.1. A-ring

3.2.1.1. One hydroxyl group

If we consider the flavones **115** and **114**, their structures differ in the position of one hydroxyl group (6 to 7). This transformation induces a weak difference of +0.04 for the EC_{50}, which means less antiradical activity, but in terms of kinetic (T_{EC50}) the time increases (from 180 to 600 minutes). The AE shows both characteristics by a drop of 46.30 to 10.15. As already showed by the TEAC assay, the 6-position is better than the 7-position for the scavenging of free radical.

3.2.1.2. Several hydroxyl groups

The introduction of a second hydroxyl group on the A-ring of the flavone (**114**) at the 5-position (**119**) or at the 8-position (**117**) shows in both cases an enhancement of the antiradical activity –0.03 and –0.07, respectively, with a better value for the introduction of the 8-hydroxyl group. The kinetic has been influenced by the introduction of none of the hydroxyl groups. Thus, the formation of the catechol at the 7,8-position seems to give to the flavone a better scavenging potential than two hydroxyl groups at the 5- and 7-positions.

We can compare now the influence of the position of a catechol on the A-ring, by studying flavones (**77)** and (**76)**. Their structures differ in the position of the catechol at the 6- and 7- positions (**77**) and 7-, and 8-positions (**76**). The EC_{50} increases from 0.11 to 0.21, while the kinetic (T_{EC50}) is unaffected. In terms of antiradical scavenging capacity the 6,7-dihydroxyflavone is better.

We can then order the positions of two hydroxyl groups on the A-ring imparting a better antioxidant activity:

$$6,7 > 7,8 > 5,7$$

At least we can compare the influence of three hydroxyl group on the A ring with the Bacalein (**79**) and its three hydroxyl groups at the 5-, 6-, and 7-positions, and the 6,7-dihydroxyflavone (**77**). The difference of EC_{50} is almost insignificant (0.01) but in terms of kinetic, the T_{EC50} increases from 360 to 600 minutes, which is quite twice more. In this case, the observation of the comportment of the AE is vivid because it rises from 15.15 to 27.78 and allows us to confirm the results of the TEAC assay, which showed the Bacalein as the best antioxidant among our flavones.

So the more hydroxyl groups the flavone possesses, the better is its antiradical scavenging potential.

3.2.2. B-ring and its substitution patterns

Despite few molecules tested on the DPPH assay, we are able to show the influence of the number of hydroxyl groups on the B-ring and the influence of the methylation of these hydroxyl groups.

Considering the flavone (**76**), if we introduce one hydroxyl group at the 4′-position of the B-ring, we obtain the flavone (**109**). In the DPPH assay it manifests itself in a decrease of the EC_{50} value (–0.10) for the same T_{EC50}: one hydroxyl group enhance the antioxidant activity. The introduction of a second hydroxyl group on the B-ring of (**109**) accentuates the decrease of the EC_{50} from 0.11 to 0.90, while the kinetic is not affected. The 3′,4′-dihydroxy moiety seems to emphasize the antioxidant character of flavones.

If we methylate the hydroxyl groups of the B-ring (flavones **117** into **93**), we can observe an increase of the EC_{50} value (+0.03), which would mean a loss of antioxidant activity, but nearby the time needed to reach the EC_{50} drops from 600 to 180 minutes, less than one third of the T_{EC50} of the 7,8,3′,4′-tetrahydroxyflavone (**117**). At least the methylated compound has a better AE (46.30) than the non-methylated one (AE=18.52).

In the TEAC assay we showed that the presence of three methoxyl groups on the B-ring does not improve the antioxidant potential of the flavones. In the examples of flavone (**102**) in comparison with flavone (**77**) the introduction of three methoxyl groups at the 3′-, 4′- and 5′-positions decrease the antiradical activity with a higher EC_{50} value (+0.04) and besides the kinetic rises from 600 to 800 minutes (one third more).

3.2.3. C-2-C-3 Double bond and 3-hydroxyl group

For this case of structure activity relationship we based our study on the Luteolin (**119**), Quercetin (**159**) and Taxifolin (**161**). The introduction of the 3-hydroxyl group (Luteolin into Quercetin) decreases the EC_{50} of –0.041 (–35%) for the same kinetics. If we use this hypothesis and apply it for the introduction of a 3-hydroxyl group on the 7,8,3′,4′-

tetrahydroxyflavone for the same kinetics, we should get a rise of the AE to 27.23. This phenomenon becomes more interesting in the case of the 7,8-dihydroxy-3′,4′-dimethoxyflavone, which presents an AE of 46.30. With the same calculation the 3,7,8-trihydroxy-3′,4′-dimethoxyflavone, should present an AE of 68.08.

We already saw in chapters 4 and 5, that the double bond between C-2 and C-3 has an essential role in the enhancement of the delocalisation of the electrons. If we compare the Quercetin (**159**) and the Taxifolin (**161**) (suppression of the double bond), the EC_{50} rises from 0.089 to 0.15, but the most spectacular is the variation of the time T_{EC50}. Taxifolin needs twice more time than Quercetin and does not reach the EC_{50} value of the Quercetin, hence the essential presence of the double bond between C-2 and C-3 in all flavonoids to enhance the antioxidant activity.

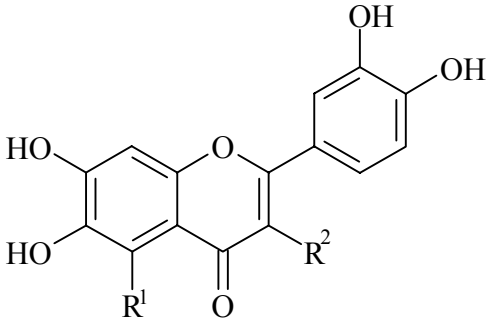
4. Conclusion

The TEAC and DPPH assays are complementary assays and allow us to say that the structural arrangements imparting greatest antioxidant activity as determined from these studies are:

- The *ortho*-3′,4′-dihydroxy moiety in the B-ring (e.g. in Luteolin, Quercetin, 7,8,3′,4′-tetrahydroxyflavone)
- The *ortho*-6,7-dihydroxy or 7,8-dihydroxy moieties in the A-ring (e.g. 6,7-dihydroxyflavone derivatives, 7,8-dihydroxyflavone derivatives)
- The di-*ortho*-5,6,7-trihydroxy moiety in the A-ring (e.g. Bacalein)
- The 2,3-double bond in combination with both the 4-keto group and the hydroxyl group in the C-ring, for electron delocalisation (e.g. in Quercetin), as long as the *o*-dihydroxy structure in the B-ring is present

- ❖ Alterations in the arrangement of the hydroxyl groups and substitution of contributing hydroxyl groups, by methylation or glycosylation decrease the antioxidant activity.

Having these informations in mind, we could suggest[155] some flavones (Scheme 75) that could show an antioxidant activity superior to the one of Quercetin.



$R^1 = R^2 = H$	6,7,3',4'-Tetrahydroxyflavone
$R^1 = OH$, $R^2 = H$	5,6,7,3',4'-Pentahydroxyflavone
$R^1 = H$, $R^2 = OH$	3,6,7,3', 4'-Pentahydroxyflavone
$R^1 = R^2 = OH$	3,5,6,7,3',4'-Hexahydroxyflavone

Scheme 75: hypothetic flavones with greatest antioxidant activity.

Chapter 7: Cosmetic and pharmaceutical applications

1. Introduction

We have shown in chapter 5, that flavonoids present a wide range spectra of UVB-absorption. That is the reason why we think that the flavonoids could be used as UV-Filter to inhibit the damages of DNA induced by UVB rays. Such studies have already been the topic of paper,[13] and our aim was to show the direct effects of flavonoids on DNA. We decided to carry out a cDNA test on the flavones of our library.

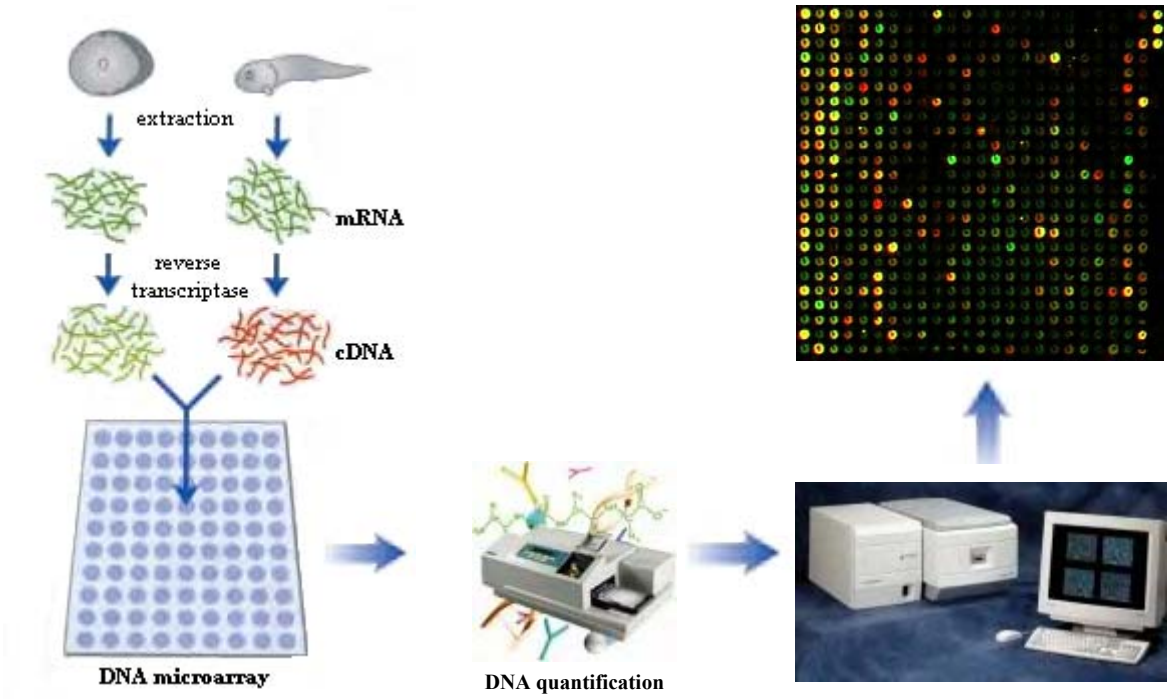
Among all the potentials of flavonoids, the most interesting applications for the industry are pharmaceutical applications. Again it has been described by many authors, and we wanted to tests all the compounds of the library in one kind of tests to show the diversity of potential of flavonoids. We selected the Protein kinases inhibitor activity to be tested at Merck.

2. Skin-focused cDNA test

Recent gene array techniques have made it possible to characterize the mRNA expression status of large numbers of genes in cells or tissues following environmental changes pathologic conditions or drug treatment. Many types of array supports can be used ranging from few immobilized sequences to dozens of thousands of genes, and using different strategies for probe labelling and detection. The macroarray support used by the BIO*alternatives* company[156] was selected because it seemed to be the most adapted system for the convenient detection of potential modulators of gene expression in skin cells. This system allows the extensive study of selected gene basal expression in different skin tissues (normal and reconstituted models) and cells and their modulation by active compounds such as retinoids. The high sensitivity and reproducibility associated to the relative simplicity of the identification of the differentially expressed genes make this system fully adapted to experimental dermato-cosmetic approaches.

2.1. RNA expression study using DNA chips

Study of RNA expression using DNA chips follows a general schema depicted in Scheme 76. DNA (oligonucleotides or cDNA) are spotted on a solid surface, to create a DNA chip. RNA targets are extracted from sample or control cells derived from tissues or culture. Extracted RNA is reverse-transcribed into cDNA probes labelled with fluorescent or radioactive reporter groups. Labelled cDNA probes are hybridised on the DNA spotted on the chip, by incubation with the array. The hybridisation data are collected as light or radioactivity emitted from the reporter groups to detect patterns of hybridisation. The RNA expression level is analysed through the intensity of the signal on each spot. The levels of RNA expression from control and from tested cells are compared.

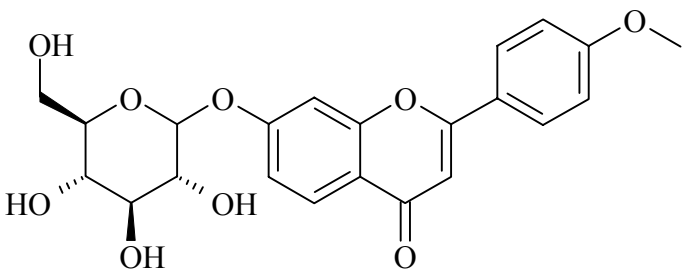


Scheme 76: General schema of the study of RNA expression using DNA chips

2.2. Data analysis

DNA chips are quite commonly used in various biotechnology domains and in our case they were used in the field of cosmetic for derm study. A membrane contains 500 to 4000 genes expressed in keratinocytes, melanocytes, fibroblasts and gene of interest in pharmacology or toxicology. Utilization of DNA chips for RNA expression study on skin

samples was compared favourably to the more classical techniques of PCR or subtractive hybridisation.[157] We let the company BIO*alternatives* test our 7-*O*-glucosyl-4′-methoxyflavone (**158**) (Scheme 77) on their membrane for a derm study.



Scheme 77: Chemical Structure of the 7-*O*-glucosyl-4′-methoxyflavone (**158**)

The response of the 500 genes on the membrane are summarised in Table 14. We can notice that only 4 genes gave a significant response to the tested flavonoid. The responses of the genes allow us to categorize the genes into two different kinds of genes: the up-regulating genes (when RE > $RE_{control}$) and the down-regulating genes (when RE < $RE_{control}$).

Gene name	Protein, function	RE (control)	RE	*%*
Beta-2-microglobulin	beta-2-microglobulin is the beta chain of major histocompatibility complex class I (HLA) molecules	11.0	19.5	***176***
Collagen 7 alpha 1 subunit (COL7A1)	Major component of dermo-epidermal junction (basal membrane)	24.7	13.9	***56***
Syndecan-4; amphiglycan; ryodocan core protein	cell surface proteoglycan that bears heparan sulfate. Syndecans-1 and -4 are induced during wound repair of neonatal skin.	6.0	15.7	***260***
Brain natriuretic peptide B (NPPB; BNP)	Peptide that could be in relation with aging and disease (marker of heart failure…).	42.5	19.3	***45***

Table 14: Gene responses of cDNA testing on 7-*O*-glucosyl-4′-methoxyflavone

The Beta-2-microglobulin, and Syndecan-4 amphiglycan ryodocan core protein are up-regulatied genes by our flavonoid. The Collagen 7 alpha 1 subunit (COL7A1) and brain natriuretic peptide B (NPPB; BNP) are down-regulatied genes in the presence of our molecule. Unfortunately they do not play a role which could be directly linked to some

cosmetic claims. Thus the 7-*O*-glucosyl-4′-methoxyflavone (**158**) was intended to be used for its good UV-absorption properties.

3. Protein kinases inhibitors tests

3.1. Definition

Phosphorylation of specific tyrosine, serine and threonine residues by protein kinases is well recognized to be involved in many cellular signal transduction pathways. Alterations in these phosphorylations are also likely to occur in oncogenesis. All protein kinases utilise ATP (adenosine triphosphate) as common substrate. Furthermore, the primary amino acid sequences (and tertiary structures, where known) in the ATP-binding region are strongly conserved among protein kinases.[158,159,160]

A large number of protein kinases (PTK) have been described. They are found in many different types of cells and are implicated in the regulation of cell transformation and cell growth, gene expression, cell-cell adhesion interactions, cell mobility and shape. Protein tyrosine kinases can be divided into two broad groups: receptor tyrosine kinases and non-receptor tyrosine kinases. The receptor tyrosine kinases possess extra cellular domains, which bind to soluble ligands, and include insulin, and growth factors receptors. The non-receptor tyrosine kinases are intracellular proteins, which may be non-covalently associated with receptor proteins.[161,162]

3.2. Protein kinase receptors

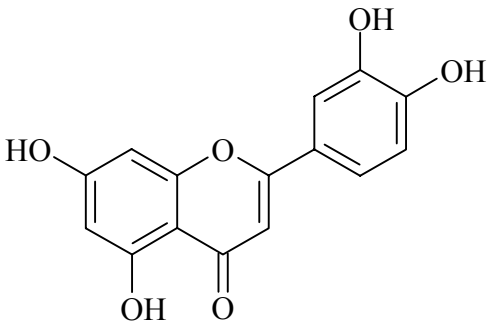
Ti2 is a tyrosine kinase receptor expressed predominantly in endothelial cells and is essential for blood vessel formation and maintenance.[163] The genesis of new blood vessels by either angiogenesis or vasculogenesis is an essential step in aetiology of many pathologies including cancer. Vasculogenesis has been implicated recently as a major contributor to tumour vascularisation. It means that the inhibition of Ti2 activity could slow the formation of tumour vascularisation and finally cause the tumour necrosis.[164]

Protein kinase B (PKB, also called Akt) is a serine/threonine kinase that plays an important role in regulation of cell proliferation and survival. PKB is activated in cells exposed to diverse stimuli such as hormones, growth factors, and extra cellular matrix components. PKB has a wide range of cellular targets, and the oncogenicity of PKB arises from activation of both proliferative and anti-apoptotic signalling. These observations establish PKB as an active target for cancer therapy.[165,166]

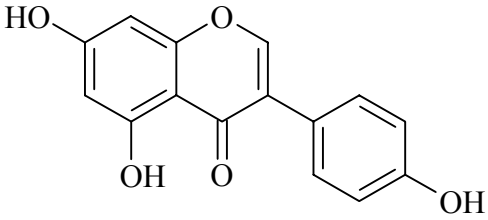
3.3. Protein kinases inhibitors

Inhibitors of these two enzymatic systems could be potential drugs candidates against cancer. The search for kinases inhibitors has uncovered classes of natural products with inhibitory activity. The identification of naturally occurring inhibitors provides important information and provides a basis for the design of additional synthetic inhibitors. In the last years, many studies have shown that several occurring flavonoids are inhibitors of protein-tyrosine kinase activity in vitro. These flavonoids, in general, acted as a competitive inhibitor of ATP binding. In most of the inactive structures, physiological inhibitors or regulatory mechanisms block the ATP binding site, indicating that steric interference with ATP binding is the major evolved mechanism to inactive the protein kinases.[167]

The lead compounds as inhibitors in bioflavonoids family are the isoflavonol genistein, and the flavonol Quercetin (Scheme 78).[168] These both compounds possess a hydroxyl group in the 5- and 7-position. The free OH in the 5-position as well as the keto-group in the 4-position is known to be important for the recognition in the enzymatic site. The substituents in the 7-position are important concerning the solubility of the compounds. Previous studies have shown that the substitution of the 7-position can afford very interesting compounds as kinase inhibitors.



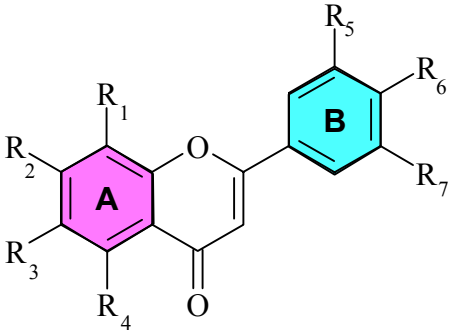
159 Quercetin



164 Genistein

Scheme 78: Bioflavonoids protein kinases inhibitors.

In the last years, compounds libraries designed to afford specific inhibitors of therapeutic target proteins have generated significant interest in drug discovery research. We have designed a flavone derivatives library to target the ATP-binding site in protein kinases (Ti2 and PKB) by combining knowledge- and diversity-based design elements. A key aspect of the approach is the identification of a molecular building blocks or scaffolds that are compatible with the binding site and therefore mimic some aspects of target specificity.[169] The tests of Ti2 and PKB are still in progress at the Pharma department of Merck KGaA to improve the kinase inhibitor capacity of our library of 26 flavones (Table 15).



N°	24	73	74	75	76	78	80	82	84	85	87	88	91	92	93	94	95	96	98	99	100	102	103	108	128	130
R_1	H	H	H	H	OH	H	H	H	H	OH	H	H	H	H	OH	H	H	H	H	H	H	H	H	H	H	H
R_2	H	OH	H	H	OH	OH	H	OH	H	OH	OH	H	H	H	OH	OH	OH	H	OH	H	H	OH	OH	H	H	H
R_3	H	H	OH	H	H	H	H	H	H	H	H	H	OH	H	H		H	H	H	OH	H	OH	H	H	H	H
R_4	H	H	H	OH	H		H	H	OH	H	OH	H	H	OH	H	H	OH	H	H	H	OH	H	OH	OH	OH	OH
R_5	H	H	H	H	H	H	H	H	H	H	H	OMe	OMe	OMe	OMe	OMe	OMe	OMe	OMe	OMe	OMe	OMe	OMe	H	H	H
R_6	H	H	H	H	H	H	OMe	OMe	OMe	OMe	OMe	OMe	OMe	OMe	OMe	OMe	OMe	OMe	OMe	OMe	OMe	OMe	OMe	OH	Cl	NH_2
R_7	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	OMe	OMe	OMe	OMe	OMe	OMe	H	H	H

Table 15: Nature of the substituents of the flavones tested as tyrosine kinase inhibitors

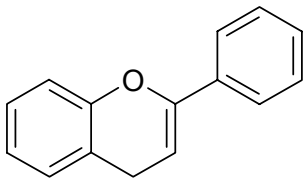
4. Conclusion

The DNA chips technology seems to be particularly well suited for the study of RNA expression, involving comparison between numerous genes and different cellular states. The diversity of choice in the sequences bound on the chip, or in the strategy of synthesis of the cDNA probes, gives endless possibilities of adaptation to very diverse domains of application. The technology of DNA chips has proved very powerful, and is now a stage of development enabling extensive applications in several domains of biotechnology. Through RNA expression studies, it will certainly contribute to future development of cosmetic ingredients.

If the results of the Ti2 and PKB tests indicate that some of the selected flavonoids have a good ability to inhibit the protein kinases, it mean that the presence of certain groups are important structural determinants that promote high affinity interactions of flavones derivatives with the tyrosine kinases and decrease interactions with protein-serine/threonine kinases. Thus, we should be able in the future to design flavonoid analogues that act as selective inhibitors of protein-tyrosine kinases.

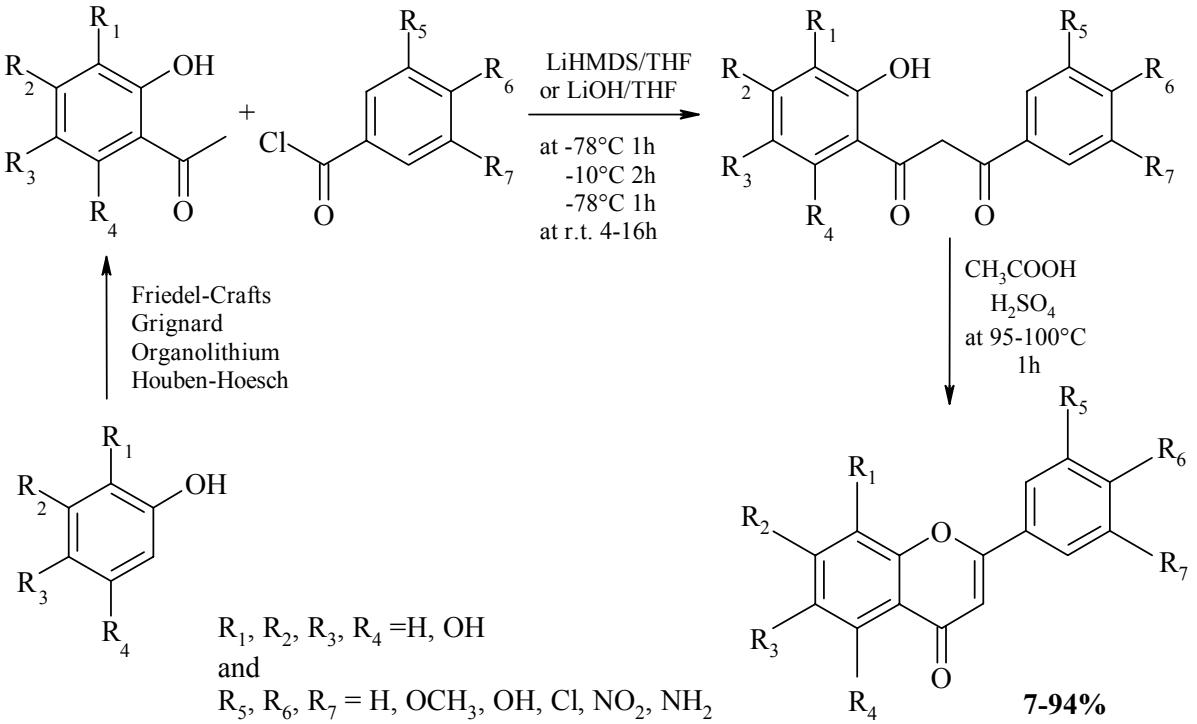
Conclusion

Flavonoids are a very large and important group of polyphenolic natural products, whose common structure is a fused aromatic heterocyclic ring system: the 2-phenylbenzopyran (Scheme 79). They were extensively studied for their various applications, and many routes to flavonoids have been described so far. We have investigated flavonoid synthesis and properties in order to study a larger set of derivatives for a better understanding their potential in different application fields, like for cosmetics use.



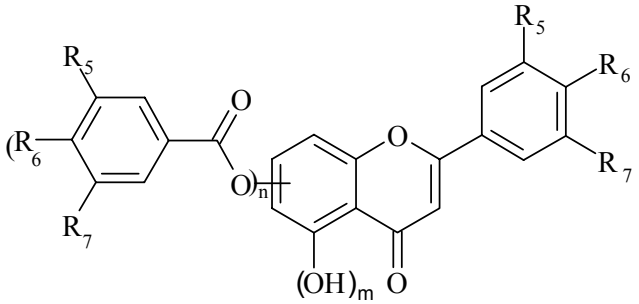
Scheme 79: chemical structure of 2-phenylbenzopyran

First, we have investigated the aldol condensation of acetophenones with aroyl chlorides. Thus, we prepared various polyphenolic substrates. The syntheses of the desired acetophenones were possible through different kind of reactions: Friedel-Crafts, Grignard, organolithium and Houben-Hoesch syntheses (Scheme 80).



Scheme 80: The Cushman and Nagarathman method

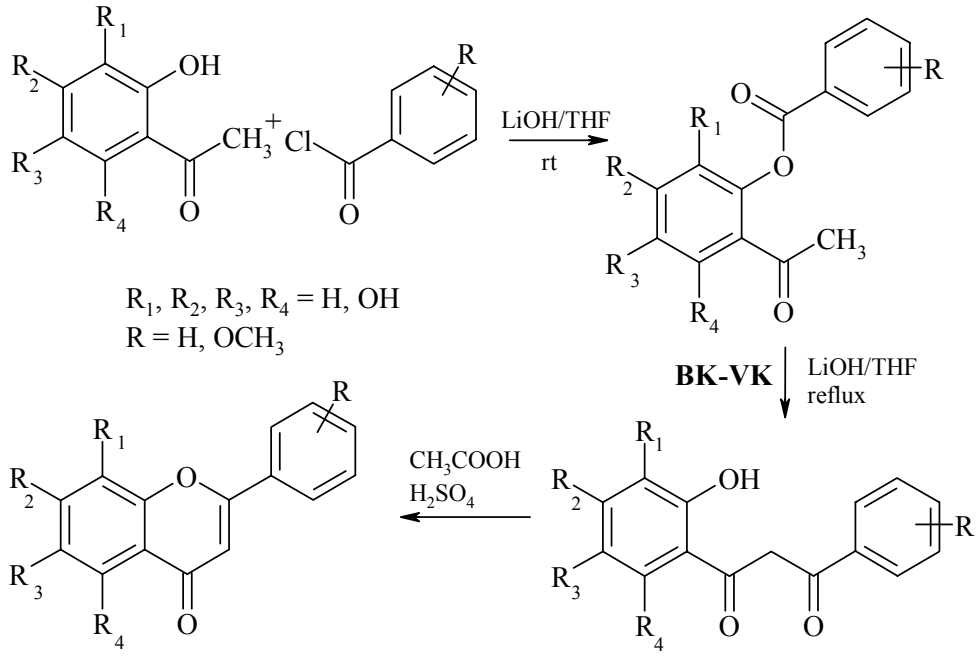
We then applied the lithium bis(trimethylsilyl)amide method developed by Cushman and Nagarathman but modified it by using lithium hydroxide as a base to furnish the 1,3-diketone intermediates, which can be cyclodehydrated in the presence of acetic acid and sulphuric acid (Scheme 80). We developed our method as a building block approach for the general synthesis of flavones by applying minor adjustments on the structure of the acetophenones and benzoyl chloride corresponding to the related flavones.



R_5, R_6, R_7 = H,OMe; n = 1,2; m = 0,1

Scheme 81: *O*-aroyl formation

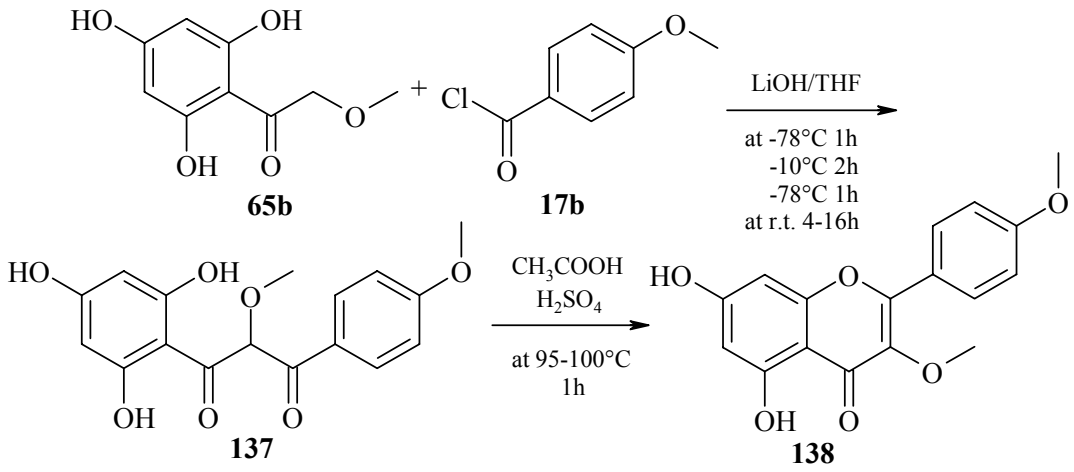
As we observed the formation *O*-aroyl substitutions of polyhydroxylated flavones (Scheme 81), in order to avoid this side product reaction we further modified the synthesis as a Baker-Venkataraman rearrangement, in which a 2-*O*-aroylacetophenone undergoes a base catalysed rearrangement to the corresponding 1,3-diketone (Scheme 82).



R_1, R_2, R_3, R_4 = H, OH
R = H, OCH_3

Scheme 82: Baker-Venkataraman rearrangement with LiOH

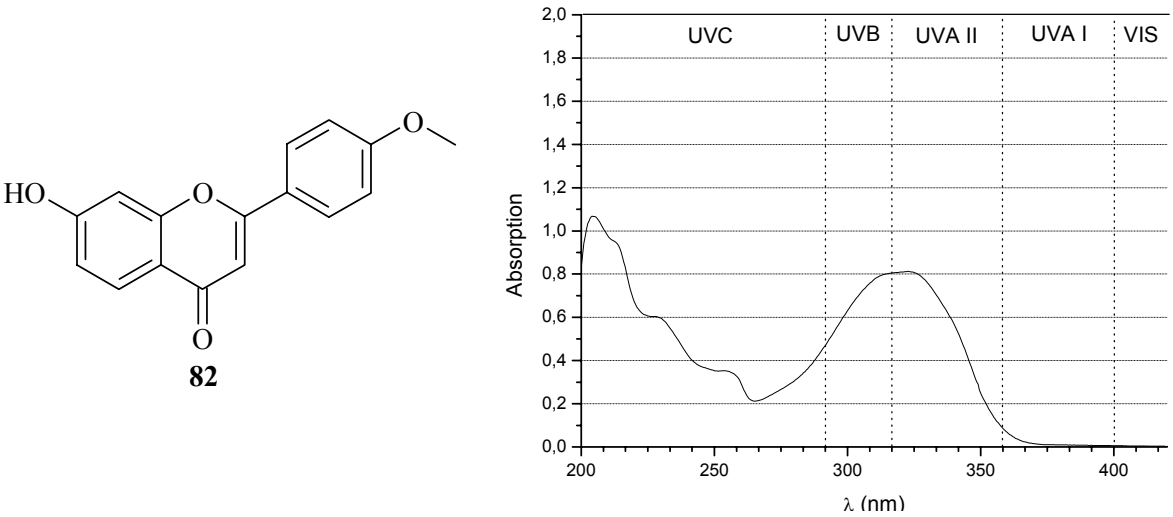
The overall compound library obtained by this strategy is composed of 48 flavones, and the synthesis has also been extended to the flavonols after the isolation of our first derivative, the Kaempherol 3,4′-dimethyl ether (**138**) (Scheme 83).



Scheme 83: extension of the synthesis to the flavonols

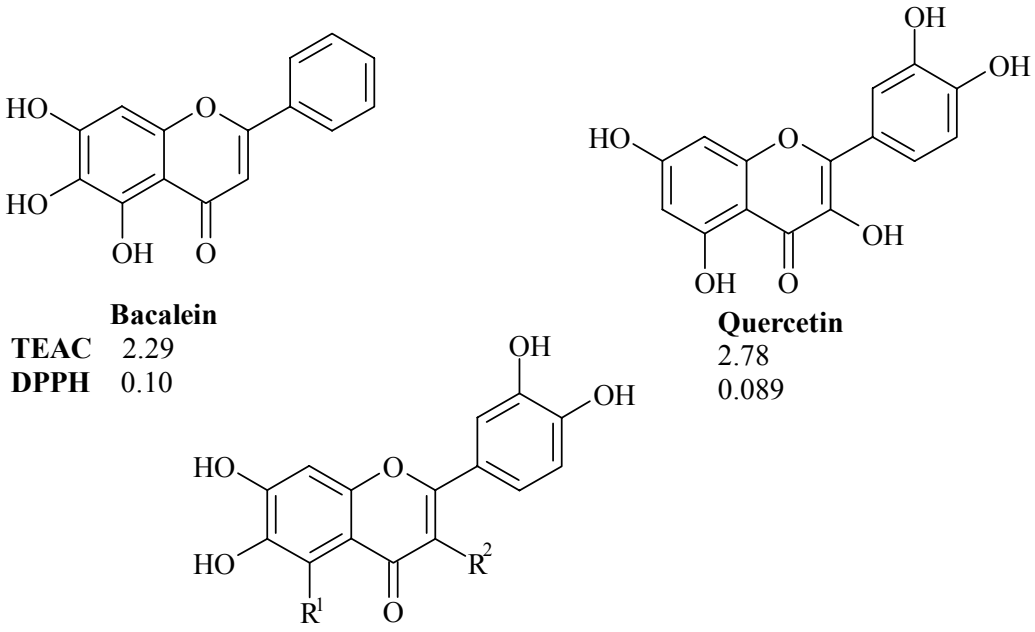
We next required a Structure-Activity-Relationship to study and better understand the potential of flavonoids (or some classes of flavonoids). The SARs were used as a directive guideline to help us in the choice of flavonoids according to their properties: the variation of the substituents (number, position on the skeleton, nature) first showed influences on the chemical shift of the carbon atoms in ^{13}C-NMR spectra, on the planar aspect of the skeleton or of its aryl-*O*-bond that leads to a change of the electron density of the compound, hence the conformation, the physical and/or chemical properties.

Then we studied the UV absorption properties, and we were able to select some structural patterns (hydroxyl groups at the 5- or 8- position, catechol at the 3′- and 4′-positions, double bond between C-2 and C-3, hydroxyl group at the 3-position) in order to build flavonoids absorbing in the UV-A and -B ranges. Among them, the Pratol (**82**) (7-hydroxy-4′-methoxyflavone) absorbs beyond 323 nm (ε 23490) and therefore could be developed as filter between UVB- and UVA II- ranges (Scheme 84).



Scheme 84: Structure and absorption spectrum of the 7-Hydroxy-4′-methoxyflavone (**82**)

The complementary TEAC and DPPH antioxidant assays were carried out to help us to establish the structural arrangements imparting the greatest antioxidant activity. We demonstrated that the catechol on the B-ring (*ortho*-3′,4′-dihydroxy moiety) like those of the A-ring (*ortho*-6,7-dihydroxy or 7,8-dihydroxy moieties), the di-*ortho*-5,6,7-trihydroxy moiety in the A-ring (e.g. Bacalein), the 2,3-double bond in combination with both the 4-keto group and the hydroxyl group in the C-ring, were some important substitution patterns.



Bacalein
TEAC 2.29
DPPH 0.10

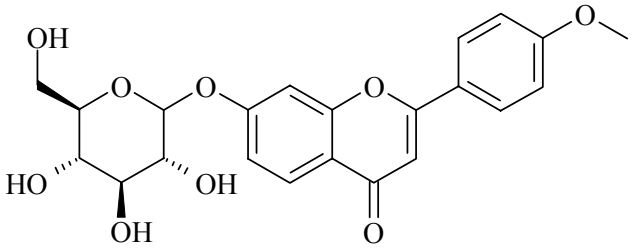
Quercetin
2.78
0.089

$R^1 = R^2 = H$ 6,7,3',4'-Tetrahydroxyflavone
$R^1 = OH, R^2 = H$ 5,6,7,3',4'-Pentahydroxyflavone
$R^1 = H, R^2 = OH$ 3,6,7,3', 4'-Pentahydroxyflavone
$R^1 = R^2 = OH$ 3,5,6,7,3',4'-Hexahydroxyflavone

Scheme 85: hypothetic flavones with greatest antioxidant activity.

If all those conditions are present within the structure of one compound, this could provide an antioxidant activity that could extend beyond the one of Quercetin (Scheme 75).

Complementary tests have been carried out on our flavonoid library using a of a DNA chip technology. This has proved very powerful in that RNA expression studies showed no significant effects of the 7-*O*-glucosyl-4′-methoxyflavone (**158**) on the genes related to the skin function. Ti2 and PKB tests were still in progress to show if our flavonoids have a good ability to inhibit the protein kinases.



Scheme 86: Chemical Structure of the 7-*O*-glucosyl-4′-methoxyflavone (**158**)

In summary, all kinds of flavonoids can be obtained in an easy manner by parallel synthesis. We will be able to design flavonoid analogues that act as optimal UV-Filters, and/or Antioxidant ingredients. In the future, we will conceive selective inhibitors of protein-tyrosine kinases and cDNA testing will enhance the source of biological activity informations.

Conclusion

Les Flavonoïdes sont un très large et important groupe de produits naturels polyphénoliques, dont la structure commune est un système de cycles aromatiques et hétérocycliques fusionnés: le 2-phénylbenzopyrane (Schéma 1). Ils ont été intensivement étudiés pour leurs diverses applications, et plusieurs routes pour les flavonoïdes ont été décrites jusqu'ici. Nous avons examiné la synthèse des flavonoïdes et leurs propriétés afin d'étudier une large palette de composés pour une meilleure compréhension de leurs potentiel dans les différents domaines d'applications, comme l'utilisation dans les cosmétiques.

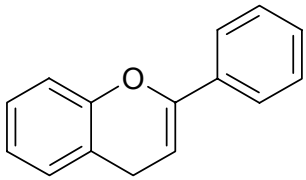


Schéma 1: structure chimique du 2-phénylbenzopyrane

Tout d'abord, nous avons examiné la condensation aldolique d'acétophénones avec des chlorures d'acides aromatiques. Ainsi, nous avons préparé des substrats polyphénoliques divers. Les synthèses d'acétophénones désirées étaient possibles par différentes types de

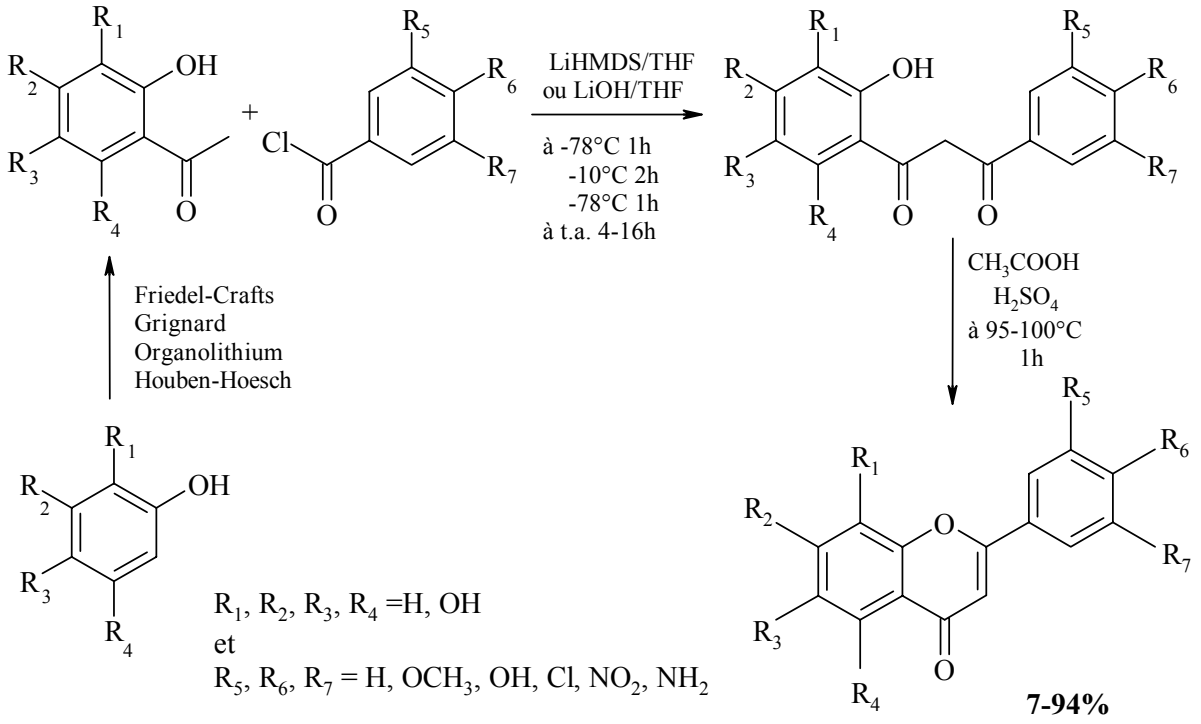
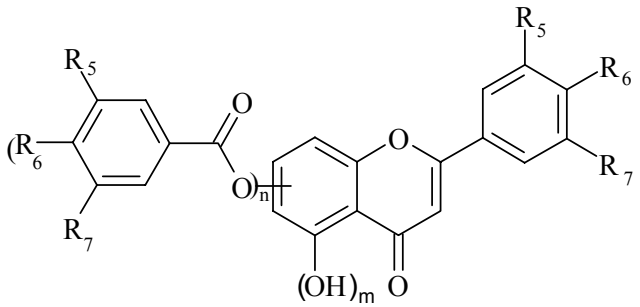


Schéma 2: la méthode de Cushman et Nagarathman

réactions: Synthèses de Friedel-Crafts, de Grignard, d'organolithiens et de Houben-Hoesch (Schéma 2).

Nous avons ensuite appliqué la méthode utilisant le lithium bis (triméthylsilyle) amide, développée par Cushman et Nagarathman, mais l'avons modifiée en utilisant l'hydroxyde de lithium comme une base pour fournir les intermédiaires 1,3-dicétones, qui peuvent être cyclisés et déshydratés en présence d'acides acétique et sulfurique (Schéma 2). Nous avons développé notre méthode comme l'approche de synthons pour la synthèse générale de flavones en appliquant des ajustements mineurs sur la structure des acétophénones et des chlorure d'acides benzoïques liés aux flavones correspondantes.



R_5, R_6, R_7 = H,OMe; n = 1,2; m = 0,1

Schéma 3: formation des composés *O*-aroyl.

Par ailleurs, nous avons observé la formation des substitutions *O*-aroyles des flavones polyhydroxylées (Schéma 3), alors pour éviter cette réaction secondaire et nous avons à nouveau modifié la synthèse en appliquant le réarrangement de Baker-Venkataraman, dans lequel une acétophénone 2-*O*-aroyle subit lors d'une catalyse basique, le réarrangement en la 1,3-dicétone correspondante (Schéma 4).

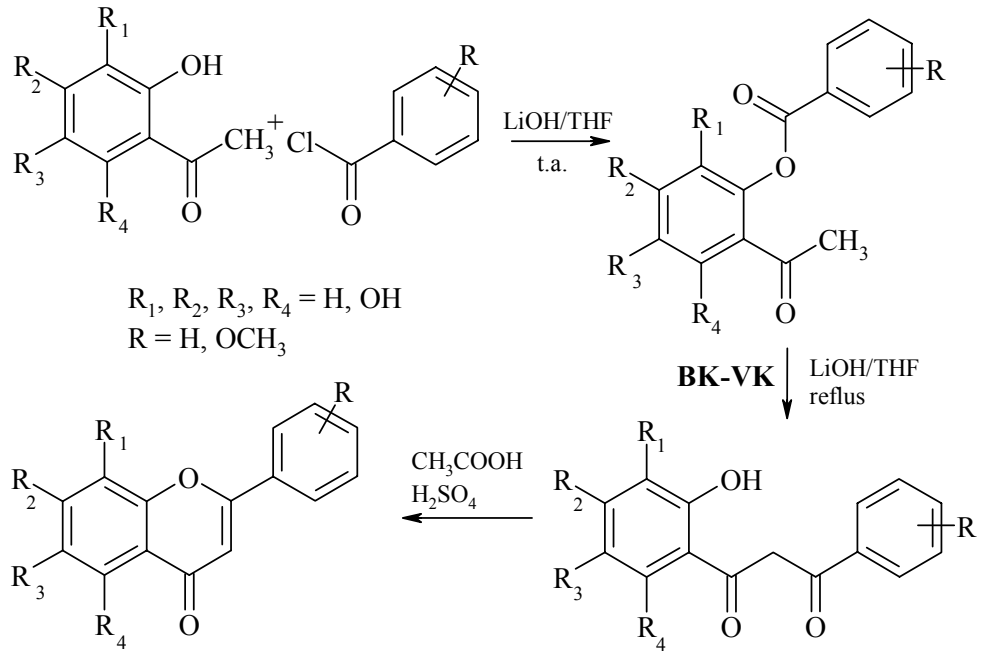


Schéma 4: réarrangement de Baker-Venkataraman avec LiOH

La bibliothèque globale de composés obtenue par cette stratégie, est composée de 48 flavones et la synthèse a également été étendue aux flavonols après l'isolement de notre premier dérivé, le Kaempherol 3,4′-diméthyle éther (**138**) (Schéma 5).

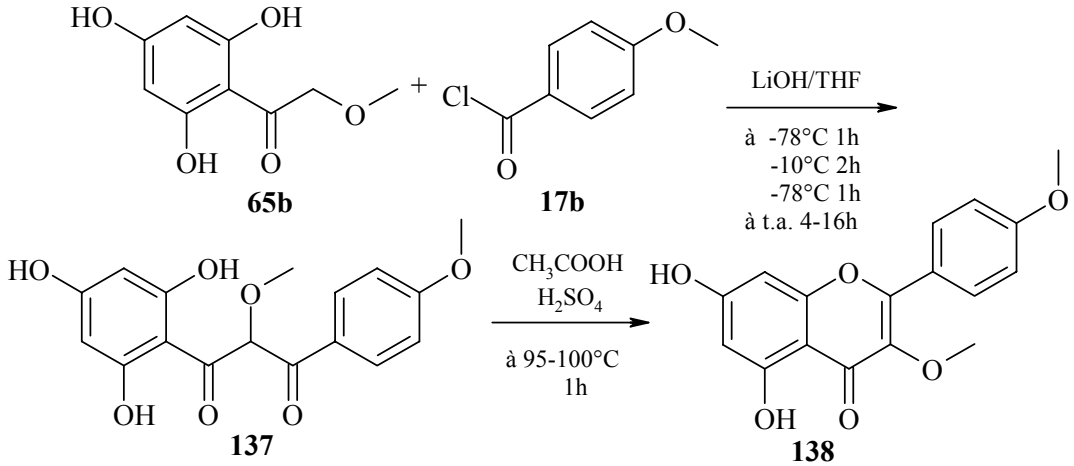
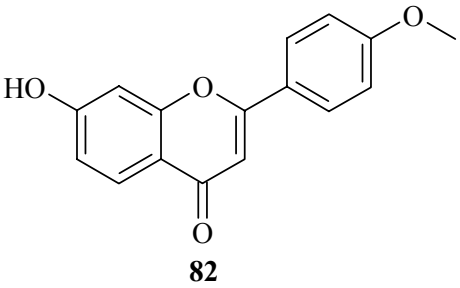


Schéma 5 : extension de la synthèse au flavonols

Il a été ensuite nécessaire d'établir un rapport entre l'activité et la structure (RAS) pour étudier et mieux comprendre le potentiel des flavonoïdes (ou quelques classes de flavonoïdes). Les RASs ont été utilisés comme une ligne directrice pour nous aider dans le choix des flavonoïdes selon leurs propriétés: la variation des substituants (le nombre, la position sur le squelette, la nature) a d'abord montré des influences sur le déplacement

chimique des atomes de carbone dans les spectres ^{13}C-RMN, sur l'aspect planaire du squelette ou de sa liaison *O*-aryle qui mène à un changement de la densité électronique du composé, et par suite la conformation, les propriétés physiques et/ou chimiques.

Nous avons, à partir des propriétés d'absorption ultra violettes, été capables de choisir quelques modèles structurels (des groupes hydroxyles en position 5 ou 8, un catéchol aux positions 3′ et 4′, une liaison double entre C-2 et C-3, le groupe hydroxyle en position 3) pour construire des flavonoïdes absorbant dans les domaines de l'UV-A et -B. Parmi eux, le Pratol (**82**) (7-hydroxy-4′-méthoxyflavone) absorbe au-delà de 323 nm (ε 23490) et pourrait donc être développé comme un filtre dans les domaines d'UVB et d'UVA-II (Schéma 6).



82

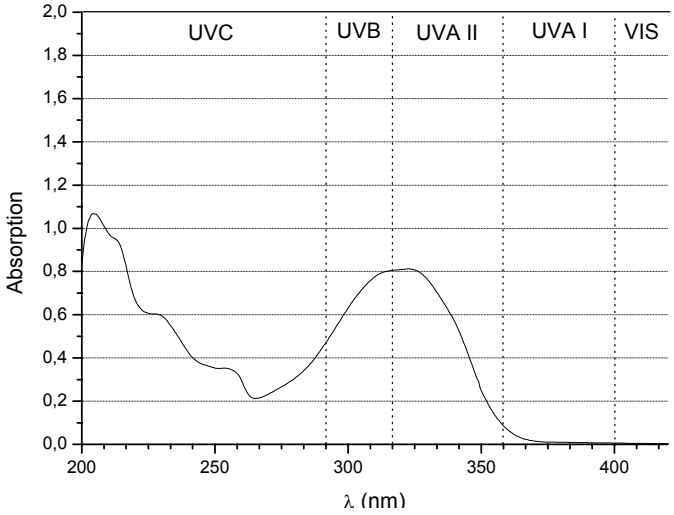
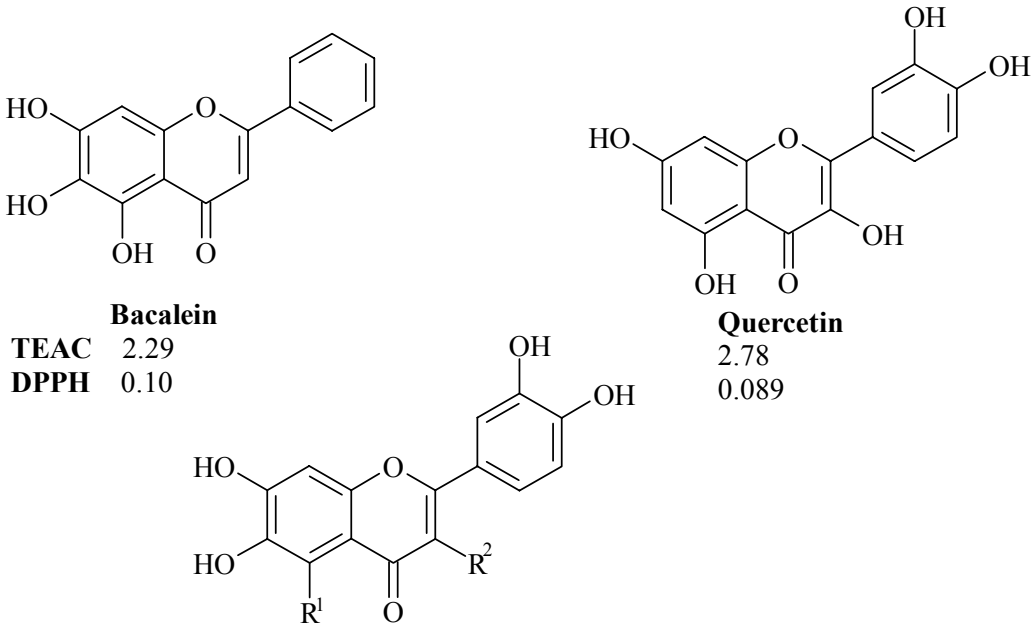


Schéma 6: Structure chimique et spectre d'absorption de la 7-Hydroxy-4′-méthoxyflavone (**82**)

Les essais antioxydants complémentaires TEAC et DPPH ont été effectués pour nous aider à établir les dispositions structurales procurant l'activité antioxydante la plus grande. Nous avons démontré que le catéchol sur le cycle B (moitié *ortho*-3,4-dihydroxy) comme ceux de du cycle A (des moitiés *ortho*-6,7-dihydroxy ou 7,8-dihydroxy), la moitié di-*ortho*-5,6,7-trihydroxy du cycle A (p. ex. Bacalein),la double liaison C2/C3 en combinaison avec le groupe cétone en C-4 et le groupe hydroxyle sur le cycle C, étaient quelques modèles de substitution importants.



Bacalein
TEAC 2.29
DPPH 0.10

Quercetin
2.78
0.089

$R^1 = R^2 = H$ 6,7,3',4'-Tetrahydroxyflavone
$R^1 = OH, R^2 = H$ 5,6,7,3',4'-Pentahydroxyflavone
$R^1 = H, R^2 = OH$ 3,6,7,3', 4'-Pentahydroxyflavone
$R^1 = R^2 = OH$ 3,5,6,7,3',4'-Hexahydroxyflavone

Schéma 7: flavones hypothétiques ayant une activité antioxydante supérieure.

Si toutes ces conditions sont présentes dans la structure d'un composé, cela pourrait fournir une activité antioxydante qui pourrait s'étendre au-delà de celle de la Quercetin (Schéma 7).

Des essais complémentaires ont été effectués sur notre bibliothèque de flavonoïdes utilisant une technologie de puces d'ADN. Elle a prouvé être très puissante dans ces études d'expression d'A.R.N. ne montrant aucun effet significatif du 7-*O*-glucosyl-4′-méthoxyflavone (**158**) sur les gènes liés à la fonction de peau. Les essais de Ti2 et PKB étaient toujours en cours de progression pour montrer si nos flavonoïdes ont une bonne capacité d'inhiber les protéines kinases.

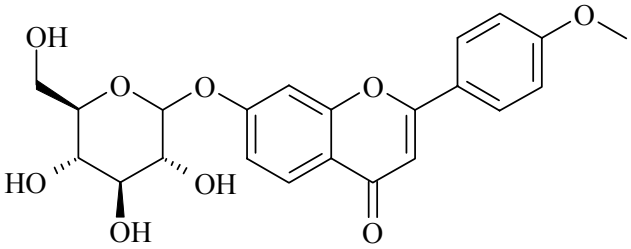
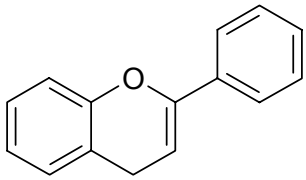


Schéma 8: Structure chimique de la 7-*O*-glucosyl-4′-methoxyflavone (**158**)

En résumé, tous les types de flavonoides peuvent être obtenues d'une façon aisée par la synthèse parallèle. Nous serons capables de concevoir des analogues de flavonoides qui agissent comme des filtres UV optimaux, et/ou des ingrédients antioxydants. Dans l'avenir, nous concevrons les inhibiteurs sélectifs de protéines-tyrosine kinases et la mise à l'épreuve des cDNA augmentera la source d'informations d'activité biologiques.

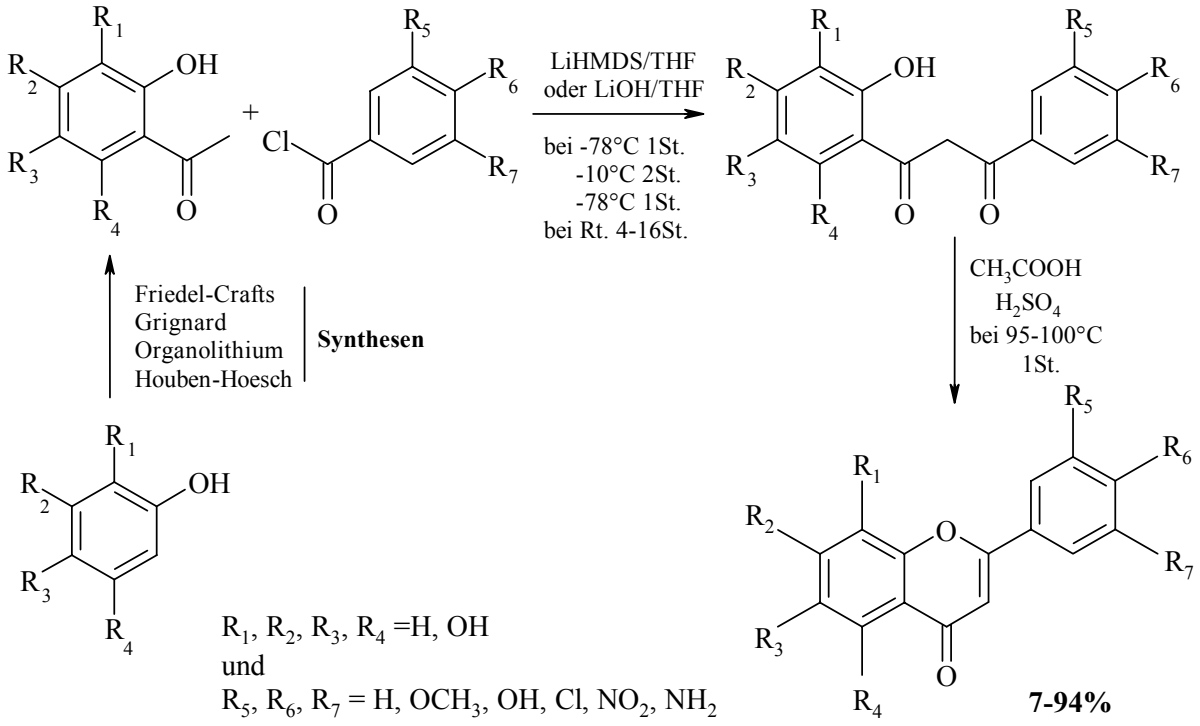
Zusammenfassung

Flavonoide sind eine sehr große und wichtige Gruppe von polyphenolischen Naturstoffen, deren allgemeine Struktur auf einem anellierten heteroaromatischen Ringsystem aufbaut, dem 2-Phenylbenzopyran (Schema 1). Sie wurden für ihre verschiedenartigen Anwendungen ausführlich studiert, und viele Synthesewege zu Flavonoiden sind bekannt. Wir haben Synthese und Eigenschaften von Flavonoiden systematisch untersucht, um einen größeren Satz von Verbindungen für ein besseres Verständnis ihres Potentials in verschiedenen Anwendungsfeldern zu studieren wie beispielsweise für den Kosmetik-Gebrauch.



Schema 1: Chemische Struktur des 2-Phenylbenzopyrans

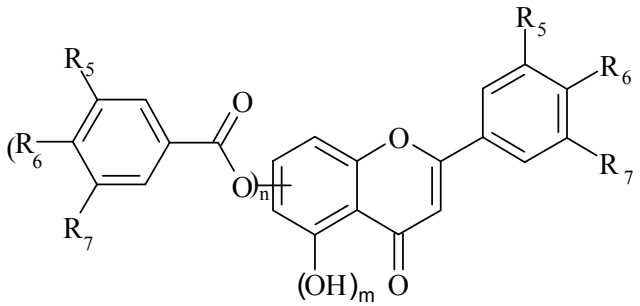
Erstens haben wir die Aldol-Kondensation von Acetophenonen mit aromatischen Carbonsäurechloriden untersucht. Zunächst stellten wir verschiedenartige polyphenolische



Schema 2: Die Cushman und Nagarathman-Methode

Vorstufen her. Die Synthese der gewünschten Acetophenone war mittels verschiedener Arten von Reaktionen möglich: Friedel-Crafts-, Grignard-, Organolithium- und Houben-Hoesch-Synthesen (Schema 2).

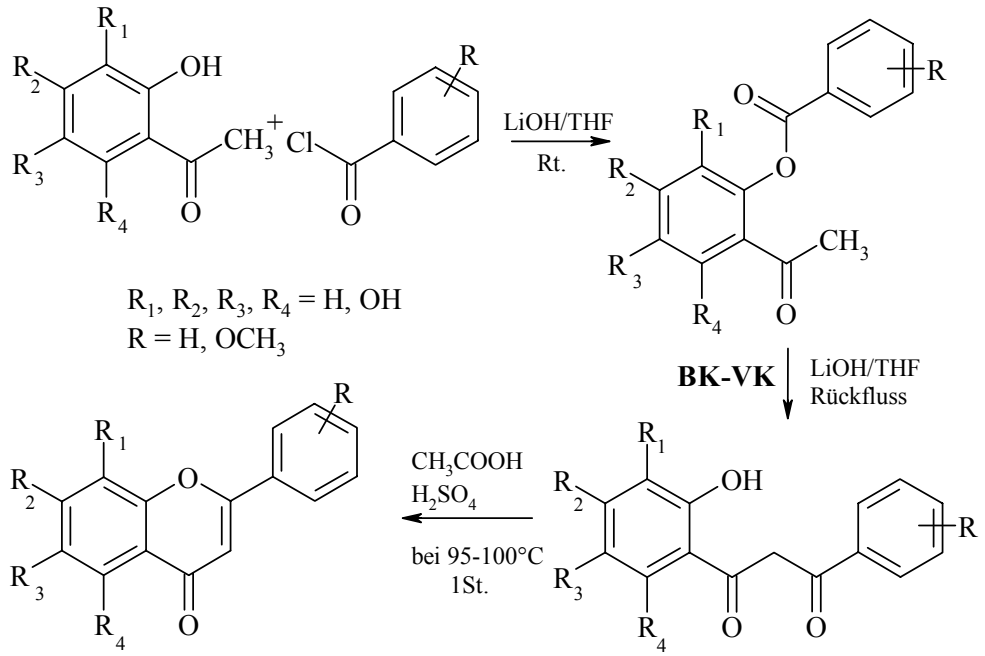
Wir wandten dann die von Cushman und Nagarathman entwickelte Lithium-bis(trimethylsilyl)amid-Methode an, aber modifizierten diese durch Verwendung von Lithiumhydroxyd als Base, um die 1,3-Diketone als Zwischenprodukte zu erzeugen, die in Gegenwart von Eisessig–Schwefelsäure (Schema 2) zyklisiert und dehydriert werden können. Es gelang, unsere Methode als eine Baustein-Synthese zu entwickeln für eine allgemeine Synthese von Flavonen, bei der nur noch kleinere Anpassungen an die jeweilige Natur des Acetophenones und des Aroylchlorids nötig sind entsprechend der gewünschten Flavonstruktur.



R_5, R_6, R_7 = H,OMe; n = 1,2; m = 0,1

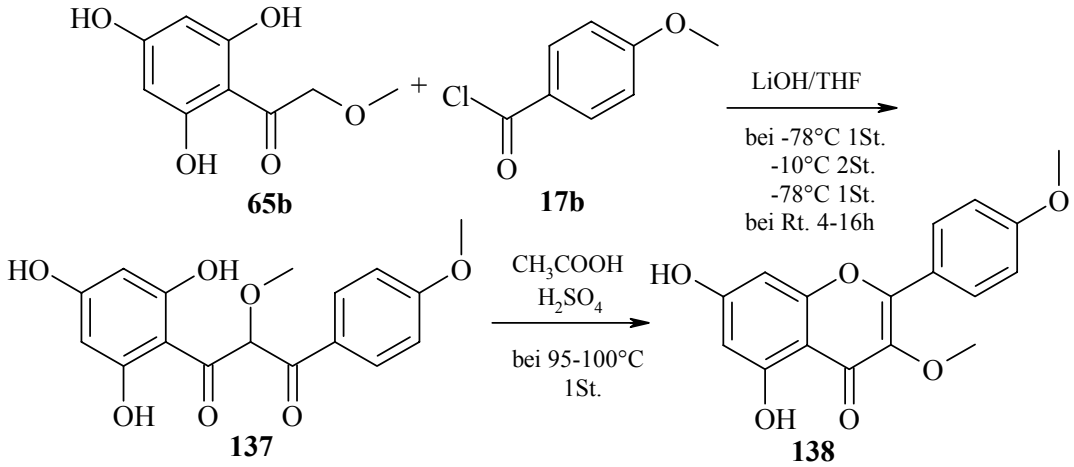
Schema 3: Bildung von *O*-Aroylderivaten

Da wir die konkurrierende Bildung von *O*-Aroylderivaten der polyhydroxylierten Flavone als Nebenprodukte beobachteten (Schema 3), modifizierten wir weiter die Synthese als eine Abwandlung des Baker-Venkataraman-Verfahrens, in der gezielt ein 2-*O*-Aroylacetophenon erzeugt wird, das dann basenkatalysiert zum entsprechenden 1,3-Diketon umgelagert werden kann (Schema 4).



Schema 4: modifizierte Baker-Venkataraman-Umlagerung mit LiOH als Base

Die gesamte, mit dieser Strategie erzeugte Verbindungsbibliothek besteht aus 48 Flavonen, wobei die Synthese auch zur Herstellung von Flavonolen wie dem Kaempherol-3,4′-dimethylether (**138**) angepaßt werden konnte (Schema 5).

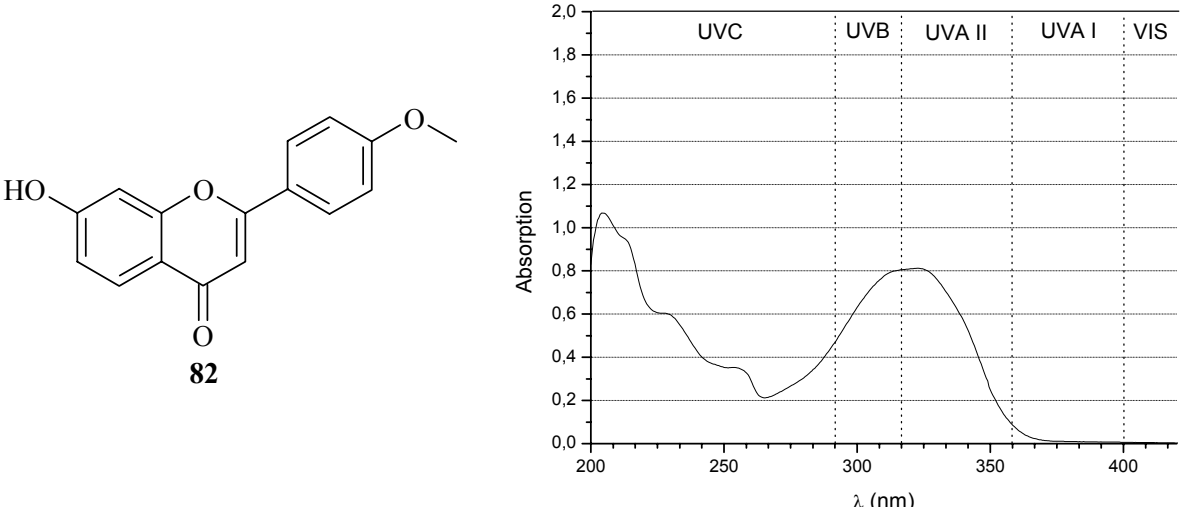


Schema 5: Erweiterung der Synthese zu Flavonolen

Als nächstes interessierte uns eine Struktur-Aktivitätsbeziehung (SAB), um das Potential von Flavonoiden (oder einzelner Klassen von Flavonoiden) besser verstehen zu können. Die SABen wurden als eine Leitlinie verwendet, um die Flavonoide nach ihren Eigenschaften einordnen zu helfen: die Veränderung der Substituenten (Zahl, Position am Skelett, Natur) liefert erwartungsgemäß im Einfluß auf die chemische Verschiebung der Kohlenstoff-Signale

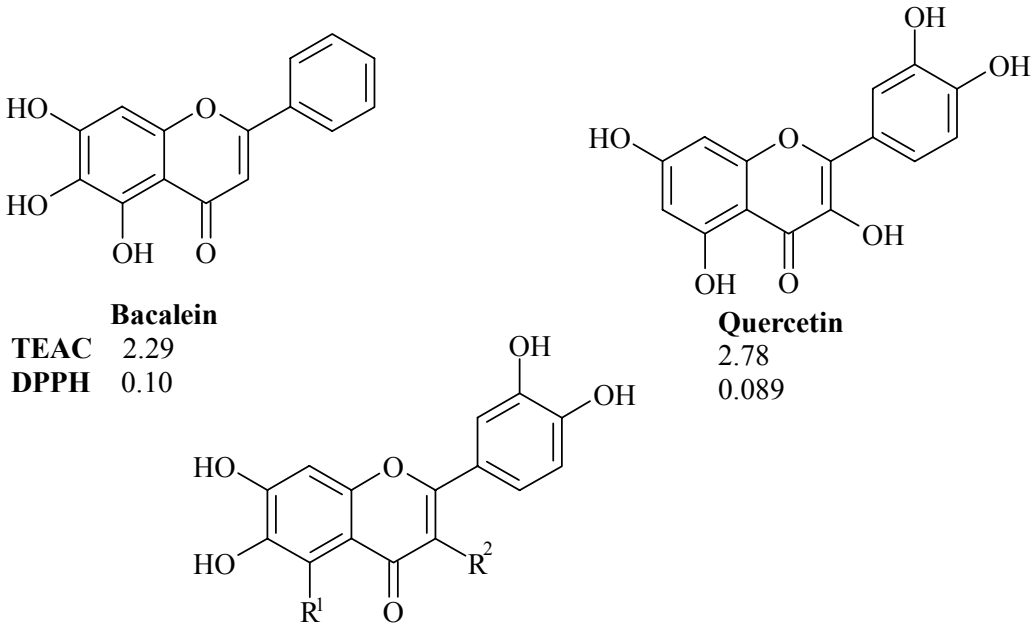
in den ^{13}C-NMR Spektren Hinweise auf die Planarität des Skelettes oder seiner Aryl-*O*-Bindung, und damit auf eine Änderung der Elektrondichteverteilung in den Verbindungen, was mit den physikalischen bzw. chemischen Eigenschaften korreliert werden kann.

Aus dem Studium der UV-Absorptionseigenschaften wurde es möglich, bestimmte strukturelle Muster (Hydroxylgruppen an der 5- oder 8-Position, Katechol an 3′/4′-Position, Doppelbindung zwischen C-2/C-3, Hydroxylgruppe an 3-Position) zu identifizieren, um gezielt Flavonoide herstellen zu können, die im UV-A und -B-Bereich absorbieren. Unter diesen absorbiert das Pratol (**82**) (7-Hydroxy-4′-methoxyflavon) oberhalb von 323 nm (ε 23490) und konnte deshalb als Filter zwischen UVB- und UVA II-Bereich entwickelt werden (Schema 6).



Schema 6: Struktur und Absorptionsspektrum des 7-Hydroxy-4′-methoxyflavon (**82**)

Ergänzende Antioxidans-Untersuchungen nach der TEAC- und DPPH-Methode wurden ausgeführt, um die strukturellen Voraussetzungen identifizieren zu können, die eine größtmögliche Antioxydans-Aktivität ergeben. Es zeigte sich, dass ein Katechol im B-Ring (*ortho*-3′,4′-Dihydroxyeinheit) und im A-Ring (*ortho*-6,7-Dihydroxy- oder 7,8-Dihydroxysubstitution) oder eine 5,6,7-Trihydroxylierung im A-Ring (z.B. Bacalein), sowie die 2,3-Doppelbindung in Verbindung sowohl mit der 4-Ketogruppe als auch mit der Hydroxylgruppe im C-Ring einige hierfür wichtige Strukturmuster sind.



Bacalein
TEAC 2.29
DPPH 0.10

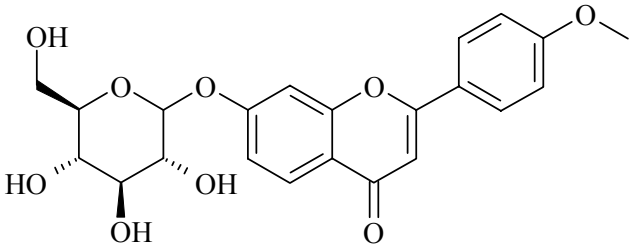
Quercetin
2.78
0.089

$R^1 = R^2 = H$ 6,7,3',4'-Tetrahydroxyflavon
$R^1 = OH, R^2 = H$ 5,6,7,3',4'-Pentahydroxyflavon
$R^1 = H, R^2 = OH$ 3,6,7,3', 4'-Pentahydroxyflavon
$R^1 = R^2 = OH$ 3,5,6,7,3',4'-Hexahydroxyflavon

Schema 7: Bekannte und hypothetische Flavone mit größter Antioxydans-Aktivität.

Wenn diese strukturellen Bedingungen innerhalb eines Flavons erfüllt sind (Schema 7), sollte das eine Antioxydans-Aktivität ergeben, die diejenige von Quercetin übertreffen könnte.

Ergänzende Tests wurden mit unserer Flavonoidbibliothek ausgeführt, die sich der DNA-Chip-Technologie bedienen. Durch die RNA-Expressions-Studien ist klar belegt, dass 7-*O*-glucosyl-4′-methoxyflavon (**158**) keine signifikante Wirkung auf die mit der Hautfunktion korrelierten Gene ausübt. Ti2- und PKB-Tests sind noch nicht abgeschlossen, die zeigen sollen, ob Vertreter aus unserer Flavonoidbibliothek in der Lage sind, Protein-Kinasen zu hemmen.



Schema 8: Chemische Struktur von 7-*O*-Glucosyl-4′-methoxyflavon (**158**)

Zusammenfassend konnten alle Typen von Flavonoiden in einfacher Weise durch eine neue Parallelsynthese erzeugt werden. Die SAR-Studien ermöglichen das gezielte Design von Flavonoidanaloga, die als optimale UV-Filter bzw. als Antioxydans-Additive wirken. Weiterhin sind selektive Hemmstoffe von Protein–Tyrosin-Kinasen in Aussicht, und cDNA-Tests werden den Zugang zu biologischen Aktivitätsinformationen verbessern.